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## TULAREMIA

[Following is a translation of selected chapters and a bibliography from a book Tulyaremia (Tularemia), edited by N. G. Olsuf'yev and G. P. Rudnev, Moscow, 1960, pages 5-23; 96-135; 207-241; 305-342; 343-381; 382-418; bibliography, 419-457.]

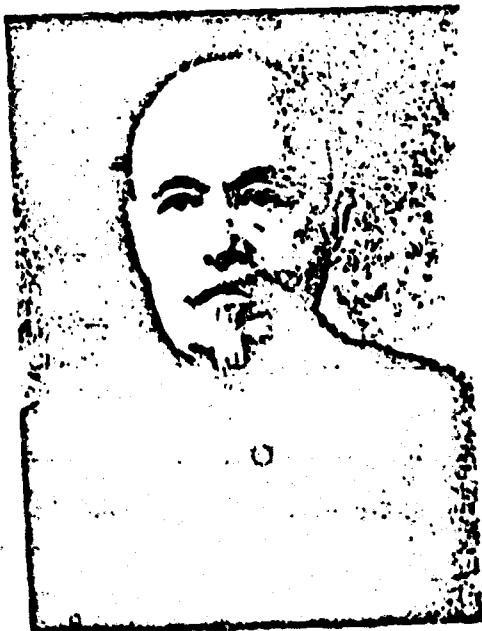
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## Chapter I

### History of the Study of Tularemia in the USSR

Tularemia was recognized for the first time on the territory of the USSR in 1926 by the physicians of the Astrakhan' Plague Station S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova (1928). Therefore, in chronological order the Soviet Union was third among the countries in which tularemia was known at that time (United States and Japan -- see Chapter II).



S. V. Suvorov



A. A. Vol'ferts

About 200 patients were detected by the authors mentioned above in several inhabited places of the Volga Delta (44-50 kilometers or more to the southeast of Astrakhan'). The form of the disease was bubonic, whereby the inguinal and femoral buboes were noted in three-fourths of those who became sick; in the others, cervical and axillary buboes were noted. The majority of patients were adolescent boys who destroyed water rats which had saved themselves from a flood and which had run into the inhabited places. Cultures of the pathogen were



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isolated which were agglutinated by convalescent serum (in a dilution of 1:800) from the buboes of three patients by passages through guinea pigs and subsequent culture on Bayle's medium (containing cystine, glucose and serum). Therefore, statements encountered in the literature to the effect that the tularemia pathogen was isolated for the first time in the USSR in 1928 by G. I. Zarkhi (N. I. Khodukin, 1951, and others) are erroneous. During laboratory work with the isolation of the pathogen four workers became sick, and their sera also agglutinated the culture obtained.

Since they did not have at their disposal the standard tularemia agglutinating antiserum and the standard strain of tularemia microbe S. V. Suvorov and coauthors called the disease which they studied a "plague-like lymphadenitis", but pointed out that there was a great similarity to a disease which had been described in North America, tularemia.

Subsequent authors had different opinions as to possible conditions of occurrence of the disease and the routes of transmission of the infection in this outbreak. G. Ya. Sinay, L. M. Khatenever and L. A. Levchenko (1936) considered it occupational. Ye. I. Novikova (1946) classified this outbreak as arthropod-borne with transmission of the infection by blood-sucking Diptera. N. I. Khodukin (1951) also considered this outbreak arthropod-borne, but the infection, in his opinion, occurred through the bites of rat fleas. The opinion of Ye. I. Novikova should be considered most correct, but the possibility has not been ruled out that some of the adolescents in destroying the rats could have become infected from direct contact (bare legs and arms) with the sick rodents. However, this was not an occupational matter, because the preparation of pelts was not carried on in the Volga delta in 1926. The predominance of inguinal-femoral buboes among the patients clearly indicates the non-occupational nature of the outbreak. Fleas should be ruled out as vectors, because they transmit tularemia through a bite with difficulty.

It would be incorrect to consider that prior to 1926 there was no tularemia in the country. Various authors have repeatedly pointed to descriptions of cases of disease among people existing in the medical literature on pre-revolutionary Russia which in retrospect could be considered tularemia. Without dealing with all the examples, we should like to dwell only on the most important ones.

In 1877, near Astrakhan' an outbreak of disease was observed accompanied by lymphadenitis; about 200 persons became sick. It was studied by the local physicians Deppner, Medovshchikov and Tavingman and in accordance with their reports was described by M. I. Galanin (1897) and others as a mild form of plague (pestis ambu-

lans). Judging by the clinical manifestations and the benignity of the course as well as the seasonality and place of occurrence these diseases should undoubtedly be considered tularemia (S. V. Suvorov and co-authors, 1928; G. I. Zarkhi, 1929; G. Ya. Sinay and coauthors, 1936; K. V. Bunin, 1953, and others), whereby the most probable route of transmission was arthropod, that is, through blood-sucking Diptera (Ye. I. Novikova, 1946). A great similarity to tularemia is found in various cases of disease observed in 1879 in St. Petersburg and in its environs and described in detail in M. I. Galanin's work (1897). The cases had a benign course and were also accompanied by external lymphadenitis. One of these cases, specifically a case in the courtier Naum Prokof'yev was considered by S. P. Botkin to be suspicious of plague, which caused a controversy at that time. In retrospect this case has been considered tularemia by many authors (D. D. Pletnev, 1932; A. F. Bilibin, 1943, and others). In 1884, in the garrison of Merv (now the city of Mary) cases of the disease were observed which were studied and described in detail by the military physician N. Voskresenskiy (1886), which, in N. I. Khodukin's opinion (1951) could be considered tularemia. However, this question remains open, because to date no natural foci of tularemia have been found in the environs of Mary, but they are known in the region of Tashauz in the same TurkmenSSR. V. A. Anishchenko (1922) in July 1921 observed a large epidemic among the inhabitants of coastal settlements of the Ob River near the mouth of the Irtysh, which, judging by the clinical characteristics of the cases described, was tularemia (G. I. Zarkhi, 1929; A. A. Miller and B. N. Stradomskiy, 1935, and others). The infection of people, apparently, occurred by the arthropod route (A. A. Maksimov, 1948). There are statements to the effect that in Armenia in 1921-1924 disease was observed similar to tularemia (V. N. Zil'fyan, 1958). M. D. Dederer (1929), in July-August 1925, detected an epidemic of lymphadenitis in some of the villages of what was formerly Ostrogzhskiy Uyesd of Voronezhskaya Oblast; thereby, the description leaves no doubt as to the tularemic (arthropod-borne) nature of his epidemic (A. A. Maksimov, 1948). In what was formerly Borisoglebskiy Uyesd of the same oblast in 1921 and 1922 a similar outbreak occurred; its nature was established in retrospect by V. S. Sil'chenko (1952).

There is firm basis for the belief that during the First World War tularemia occurred in part of the cases under the diagnosis of Volhynia or trench fever (L. V. Gromashevskiy, 1947; A. I. Volkov, 1948; A. A. Maksimov, 1948, and others). We believe also that in West Siberia the summer cases of tularemia of arthropod origin accompanied by the formation of ulcers and lymphadenitis on the body could have partially gone under the diagnosis of anthrax prior to that, A.A.

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Maksimov adheres to the same opinion (1959).

Many travellers who visited this country in the 18th and 19th centuries (I. Fal'k, A. F. Middendorf and others) have written about the mass spread of "Siberian ulcer" [anthrax] in many regions of the West Siberian Plain and the connection of this disease with transmission by blood-sucking Diptera. The fact deserves attention that the local population considered water rats and hares "unholy" animals and did not hunt them. The name "Siberian ulcer" indicates the typical nature of the ulcerative process in this disease, which with the cutaneous route of infection is equally characteristic of anthrax and tularemia. It is well known that in West Siberia cases of arthropod-borne tularemia are encountered much more often than those of arthropod-borne (that is, transmitted by vectors) anthrax, whereby arthropod-borne tularemia outbreaks are very characteristic specifically of the extensive boggy plains of West Siberia which abound in blood-sucking Diptera.

In the study of the old medical literature a certain degree of caution should be shown in evaluating various cases of disease accompanied by the formation of buboes as tularemic. Inflammation of the lymph nodes in combination with the benign course of the disease and the absence of contagiousness can be observed in a number of infectious diseases, including listerellosis, tuberculosis, etc., without mentioning the ordinary lymphadenitides. Along this line we should have a critical attitude, for example, toward army physician Chernobayev's diagnosing as tularemia cases observed in 1825 in Volynskaya Guberniya. In considering these cases tularemia, M. P. Pokrovskaya (1940) writes that "120 persons became sick" and "in all the patients there was a fever and the glands were swollen". Mention of the 120 cases with lymphadenitides is based on a misunderstanding. In Chernobayev's article (1836), in a footnote on page 130 from where this information comes, a total of six soldiers with inguinal buboes terminating in suppuration was noted. The cases occurred in the spring. The appearance of buboes was noted only in part of the soldiers and only after they were hospitalized, whereby the author associates the occurrence of the buboes with the unsanitary conditions in the pot-house, where the patients were placed because of a lack of space in the infirmary. The author does not report any other clinical signs on the basis of which one can judge the nature of these cases.

We believe also that the disease in the well-known zoologist B. S. Vinogradov which in 1921 followed the bite of a water rat on the dorsal surface of the hand was considered retrospectively tularemia without adequate basis by various authors (G. Ya. Sinay and coauthors, 1936; N. I. Khodukin, 1951, and others). Usually after the bite of an animal sick with tularemia the ulcerative-bubonic form of tularemia

develops. In a quite detailed description made by Vinogradov himself (1922) we do not find either general signs of the disease or indications of an enlargement of the regional lymph nodes. The author reports a tumor in the area of the bite which showed itself shortly after and remained about two weeks and then pain on flexion of the fingers, but this resembles more the clinical picture of erysipeloid, with which water rats are very often infected.

In 1927-1928 Pushnogostorg [State Fur-Trade Administration] in various parts of the country began large-scale procurement of water rat pelts, which involved a rapid development of the industry based on this animal. For example, about 4,500,000 rat pelts were obtained in two years in Ural'skaya Oblast alone (G. I. Zarkhi, 1929). In places where there was an active water rat industry mass cases of diseases among people occurred, and this immediately attracted the attention of physicians.



D. A. Golov

In 1927 cases of this kind among people (75 cases) were observed by V. P. Ponomarev and D. A. Shain in Mirimovskiye Yurty of Tobol'skiy Okrug (on the shore of the Irtysh), whereby the authors, independently of S. V. Suvorov and his co-workers, noted a similarity

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of the disease to tularemia (G. I. Zarkhi, 1927). According to I. N. Shukov (1928) and G. A. Shuster (1930), in the same year cases associated with the water rat industry were also observed on the Irtysh River in the north of Omskaya Oblast and on the Tavda River.

In 1928, a considerable incidence of tularemia was observed among persons engaged in the water rat industry at the beginning of the summer in what were formerly Orenburgskaya and Ural'skaya guberniyas on the Ural River, about 100 cases (D. A. Golov, A. N. Knyazevskiy, V. A. Bernikov and V. Ye. Tiflov, 1928); in what were formerly Kasimovskiy and Spasskiy uyezds in Ryazanskaya Guberniya on the Oka River -- more than 800 cases (A. A. Vol'forts, 1928; I. P. Gauzner and A. V. Belitser, 1928; L. M. Khatenever, 1930); and in Tobol'skiy Okrug on the Ob River and its tributaries -- more than 100 cases (G. I. Zarkhi, 1929). Cases of tularemia were also found in 1928 on the Lena in the environs of Yakutsk (S. B. Dubrovinskiy, 1930). Judging by the time of their occurrence (from the middle of June to the beginning of September) and by the absence of any statements of the existence of a water rat industry, these cases apparently should be considered of the arthropod-borne type.

In 1928, while studying the outbreak of disease on the Ural River mentioned above, D. A. Golov and coauthors isolated cultures of the tularemia microbe from water rats by passaging through guinea pigs and white mice (with subsequent subculture on coagulated egg yolk medium). Thereby, for the first time, the significance of water rats as sources of tularemia infection was proved accurately.

G. I. Zarkhi (1929), investigating people sick with tularemia in 1928 in what was formerly Tobol'skiy Okrug (the village of Muzhi in Obdorskiy Rayon) isolated a culture of the pathogen which was agglutinated by convalescent serum from the pus of a bubo of one patient by passage through a guinea pig. Judging by the description, the occurrence of disease was associated not only with the water rat industry but also in some cases with the transmission by blood-sucking insects. During the course of experimental work with the culture isolated Zarkhi, in 1928, became infected and became sick with tularemia. With the aim of obtaining confirmation of the diagnosis he sent his own serum to McCoy in Washington and, according to the latter's conclusion, it agglutinated American strains of the tularemia microbe up to a titer of 1:640. McCoy also isolated a strain of tularemia bacteria from a guinea pig which had been inoculated with splenic tissue (from a guinea pig) obtained at the same time from Zarkhi (Simpson, 1929). In exchange Zarkhi received a strain (No 33) of American origin from McCoy and, thereby, was able to identify his own strain, isolated from a patient in Obdorskiy Rayon (G. I. Zarkhi, 1930) with it. As a result,

Zarkhi determined once and for all that tularemia in the United States and "tularemia-like", "plague-like" or "influenza-like" diseases in the USSR are the same. In 1929, continuing the study of tularemia in Tobol'skiy Okrug, Zarkhi isolated cultures of the pathogen of this disease from water rats and the common hamster. Then the author, for the first time, studied and suggested the thermoprecipitation reaction for the diagnosis of tularemia in rodents.

In 1929 and 1930 cases of tularemia in people engaged in the water rat industry were noted in Kargatskiy Rayon of Novosibirskaya Oblast, whereby in some patients the ocular form was present which had a course similar to Parinaud's conjunctivitis. The tularemic etiology of these cases was first shown in the USSR by L. D. Mitskevich (1931). Outbreaks of tularemia associated with the water rat industry were then observed in 1930 on the Belaya River in Bashkirskaya ASSR (L. M. Khatenever, 1934) and in other places of the USSR, and were given the name of "occupational" (G. Ya. Sinay, 1934).

In the spring of 1930, at a canning plant in the city of Kurgan (West Siberia) cases of tularemia were noted among workers which were associated with the dressing of frozen hare carcasses (probably, white hares). Cases of the same origin recurred at the plant in the spring of 1931 and at the beginning of 1932 (I. F. Berezin, 1931; 1934). They were detected also among the dining room personnel, where the salted hare carcasses had been brought. Therefore, Berezin, for the first time in the USSR, established a new source of tularemia infection -- hares -- known prior to that time only in the United States and Japan.

In the summer of 1930 an outbreak of tularemia was demonstrated in Ushtobinskiy Rayon of Taldy-Kurganskaya Oblast (Southeast Kazakhstan) by P. P. Popov and G. Ya. Sinay, but in contrast to the epidemic outbreaks previously studied by Soviet investigators it was entirely associated with transmission of the infection by blood-sucking Diptera. G. Ya. Sinay (1934) proposed the name "spontaneous" for outbreaks of this kind, but afterwards this term was replaced by "arthropod-borne" as being more in accordance with the essence of the matter (N. G. Olsuf'yev, 1939). An outbreak of tularemia of similar origin was observed in the same year, 1930, by V. I. Kamanin in what was formerly Barabinskiy Okrug of Novosibirskaya Oblast and was studied by A. Ya. Krol' (L. Rabin, 1931; A. Ya. Krol', 1933). During the course of experimental work with cultures isolated from sick people, A. Ya. Krol' became infected with tularemia and died from a superimposed specific pneumonia (Ye. P. Pakhotina-Krol', 1933). Foreseeing a possible fatal outcome of his disease, A. Ya. Krol' left his body for autopsy and study, believing that this would be the first

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case of investigation of pathological changes in men with tularemia in the USSR. All this is evidence of the high sense of duty and courage of Aleksandr Yakovlevich Krol', whose name should occupy a worthy place in the history of the study of tularemia in the USSR.

It should be pointed out that prior to the introduction of inoculations with living vaccine into practice practically all persons working in the laboratory with the pathogen, particularly in the autopsy room or in the vivarium with experimental animals, sooner or later became infected with tularemia, despite the careful use of precautionary measures, including work in a full plague suit. The infection usually occurred by aspiration (G. Ya. Sinay, 1940), which was indicated by the characteristics of the clinical course of the disease in cases described in detail in the literature (D. A. Golov and coauthors, 1928; G. I. Zarkhi, 1929, and others) as well as in many others of which we know personally.

The initial period of study of tularemia described, which encompassed the period of time from 1926 through 1930, is characterized by the accumulation of the original information about the distribution of this disease on the territory of the USSR as well as about its etiology, epidemiology and clinical aspects. At that time, the considerable spread of cases of the occupational type among certain population groups depended not only on the mass nature of the water rat industry but apparently also on its newness, as the result of which a large number of non-immune groups, particularly among adolescents, were involved in the industry. Undoubtedly also in 1926-1930 a considerable increase in the water rat census was observed in many places of the USSR accompanied by the development of acute tularemia epizootics among their population. This is indicated not only by the numerous cases during work in the water rat industry but also the occurrence of a number of arthropod-borne outbreaks.

The period under analysis coincided with the general active development of medical science in the young Soviet Republic, with the creation of a system of scientific research institutes of microbiological category, etc., and this contributed to making the correct diagnosis of tularemia, which had previously gone unrecognized. It is quite natural that both in the initial and subsequent periods Soviet investigators gave considerable attention to achievements in the study of tularemia by foreign authors and chiefly the data of the United States. However, quite quickly in the USSR original trends were outlined in investigations on tularemia, first in the epidemiology and epizootology of this disease and later in the clinical aspects, laboratory diagnosis, immunology, and others.

Even during the initial period of study of tularemia Soviet

authors gave special attention to the development of laboratory methods of diagnosis of tularemia. Along with isolation of pure cultures of the pathogen from people sick with the disease, which, considering its biological characteristics, was far from being a simple task technically at that time, the agglutination test was used extensively, and its high degree of specificity was confirmed (A. A. Vol'ferts, D. A. Golov, G. I. Zarkhi, L. M. Khatenever, G. Ya. Sinay, and others). However, a defect in this reaction, which consisted of late detection of agglutinins in patients, even at that time stimulated Soviet investigators to a search for a faster method of diagnosis. In world practice there was no such method. In 1931, V. A. Bychkov and L. G. Rappoport, on the basis of thorough experimental studies, suggested the allergic skin test for this purpose using a preparation which they had developed and which they called "tularin". V. N. Fedorov and Kh. Gol'dshtern (1934) as well as L. M. Khatenever, A. A. Vol'ferts, Ye. I. Novikova and Ye. D. Polumordvinova (1935) then showed the high diagnostic value of this reaction in sick people, including its value for the early diagnosis of the disease (from the fourth to the fifth day), associated with great simplicity of application. In Soviet public health practice the tularin test in combination with the agglutination test has become quite widespread not only for diagnosis of the disease but also for the retrospective detection of cases of tularemia and subsequently for the determination of immunity in those inoculated (see Chapters VIII and X). Methods were worked out for isolating the tularemia pathogen from rodent organs and other objects investigated as well as methods of obtaining pure cultures, identifying them and keeping them in a museum.

The period from 1931 through 1941 should be considered the second period in the study of tularemia. During this period, along with organization of the first specialized laboratories for the study of this infection and control of it in the country, general physicians began to become acquainted with this disease (prior to this only a limited group of specialists in scientific research institutions knew about tularemia) as the result of publication of a number of articles on tularemia in general medical journals. The development of the main methods of laboratory diagnosis of tularemia were completed (see above), and the incorporation of them into practice was begun. All this afforded the possibility of continuing the detection of the distribution of tularemia on the territory of the country as well as beginning the planned and detailed study of the clinical aspects of this disease, its epidemiology, microbiology, pathogenesis, epizootology and prophylaxis.

The first laboratory for the study of tularemia was created at the end of 1929 at the Institute of Microbiology of the Narkomzdrav RSFSR. In 1931 it was given over to the Scientific Testing Institute



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imeni L. A. Tarasevich, and in 1937 it was included in the All-Union Institute of Experimental Medicine (VIEM) imeni A. M. Gor'kiy. In 1945, the tularemia laboratory was given over to the Institute of Epidemiology and Microbiology imeni Academician N. F. Gamaleya of the Academy of Medical Sciences USSR (for purposes of economy of space in the subsequent presentation this Institute will be called IEM im. Gamaleya), a part of which it remains to date. From the time of its organization the tularemia laboratory was headed by L. M. Khatenever, who directed it until his death (1948). The co-workers of L. M. Khatenever were first G. Ya. Sinay, L. A. Levchenko and I. F. Pashkevich, and subsequently B. V. Voskresenskiy, Ye. M. Tsvetkova, O. S. Yemel'yanova, M. V. Afanas'yev, Ye. N. Tolstukhina, P. N. Burgasov, I. N. Mayskiy, and others.

This laboratory and L. M. Khatenever himself played a great part in the study of tularemia in the USSR. Under his supervision investigations were made on epidemiology, microbiology, immunology, therapy and prophylaxis of tularemia, and diagnostic and therapeutic bacterials were also studied and prepared (tularin, diagnosticum, therapeutic vaccine, etc.). L. M. Khatenever gave great attention to the popularization of knowledge about tularemia: the main textbooks on this infectious disease were written -- with his participation (G. Ya. Sinay, L. M. Khatenever and L. A. Levchenko, 1936) or by himself (L. M. Khatenever, 1942), or else were published under his editorship (L. M. Khatenever, 1943).

In 1934 the tularemia department was organized at the "Mikrob" Institute at Saratov. The various workers of this Institute (D. A. Golov, V. A. Berdnikov, Ye. V. Tiflov, V. N. Fedorov, A. A. Vol'ferts, A. A. Flegontova, and others) made studies on tularemia even before the organization of the department, beginning with 1928. A. A. Vol'ferts was at the head of the department; her co-workers were N. A. Popov, N. K. Vereninova, L. K. Denisenko, and others.

The department studied problems of microbiology, immunology, epidemiology, epizootology, parasitology, diagnosis and serotherapy of tularemia and also produced diagnostic tularemia preparations. A. A. Vol'ferts suggested the percutaneous method of application of tularemia (1934). She published the first reviews of the literature on tularemia (1927, 1935). A. A. Vol'ferts almost simultaneously with G. P. Rudnev (see below) suggested a more complete clinical classification of tularemia than Francis did (United States) with the inclusion of the pneumonic form (A. A. Vol'ferts, 1935).

A number of studies on the epidemiology, epizootology, clinical aspects and diagnosis of tularemia made both at the "Mikrob" Institute itself and at the Astrakhan' and Stalingrad Plague Stations

subordinate to it and in their departments (Ye. I. Novikova, G. A. Lalazarov, V. M. Tumanskiy, Z. I. Kolesnikova, V. I. Gorokhov, A. L. Kazantseva, Ye. A. Usov and others) refer to this period. V. N. Fedorov and V. F. Sivolobov (1935) then made the first study of mosquitoes as vectors of tularemia, and M. M. Tikhomirova, R. I. Timofeyeva and N. V. Ekstrem (1935) suggested a simplified agglutination test by the thick drop method.

Independently of the "Mikrob" Institute but almost at the same time V. P. Bozhenko (1935, 1936) took up the study of blood-sucking Diptera and bugs as vectors of tularemia. Beginning with 1932 D. A. Golov at the Alma-Ata Plague Station, to which he was transferred, began the planned study of problems of the epizootology of tularemia.



Professor L. M. Khatenever

For the first time in the USSR he (along with V. N. Fedorov) established the important role of ixodid ticks as vectors of tularemia by means of very thorough field and experimental studies. D. A. Golov made a detailed study of the characteristics of tularemia epizootics among water rats for five years (1932-1936) in the small spring rivulets in the environs of Alma-Ata. This type of focus, as was made clear subsequently, was more widely distributed and was given the name of mountain-valley focus (V. P. Bozhenko, 1950) or foothill.

brook (A. D. Lebedev, 1953). With the assistance of D. A. Golov, in 1933-1936, O. A. Dudinov studied experimentally the ophthalmic form of tularemia in rabbits. The results of this work were then generalized on by Dudinov in a doctoral dissertation. This was the first dissertation in the USSR on tularemia; it was defended at Tashkent in 1940.

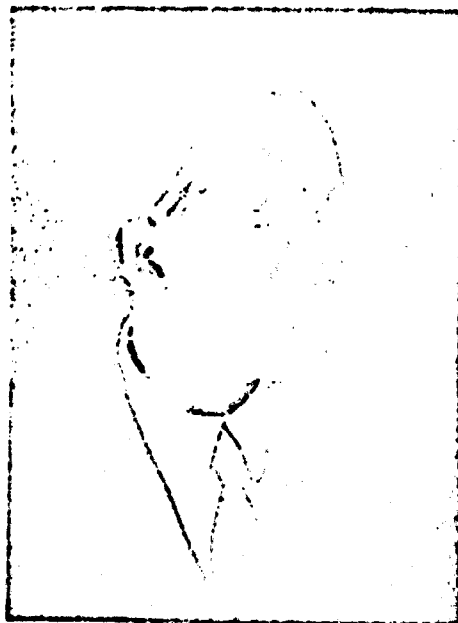
In 1934-1936 D. A. Golov and we (during periodic trips to Alma-Ata) made a large series of experimental studies for the elucidation of the role of blood-sucking Diptera in transmission of tularemia. These works were begun at the suggestion of Ye. N. Pavlovskiy, whose co-worker we were in the department of parasitology of VIEM imeni Gor'kiy (Moscow). The studies on tularemia vectors were continued in 1937 by us in cooperation with G. A. Lalazarov in the Volga delta near Astrakhan', and in 1938 in Mikhnevskiy Rayon of Moskovskaya Oblast in an expedition headed by B. V. Voskresenskiy (see below). For the purpose of assuring the continuation of these studies an office of parasitology was created in 1939 at the tularemia laboratory of the VIEM, of which we are the heads. Ye. N. Tolstukhina was a co-worker in this office during that period. This office was restored after the Second World War as a laboratory of vectors of particularly dangerous infections in the department of parasitology mentioned, which at that time was included in the IEM imeni Gmaleyeva. The trend of the works of this office and then of the laboratory was not only parasitological but also epizootological. After L. M. Khatenever's death (1948) the laboratory of vectors of particularly dangerous infectious diseases and the laboratory of tularemia were combined into one laboratory, and supervision of the laboratory was given to us. During this and the subsequent period the workers in the laboratory were T. N. Dunayev, O. S. Yemel'yanova, Ye. M. Tsvetkova, later V. G. Petrov, G. P. Uglovoy, R. A. Savel'yeva and others.

In 1934, in Rostov-na-Donu on the initiative of A. A. Miller the department of tularemia was created at the Azov-Black-Sea Regional Institute of Microbiology and Epidemiology. The department was headed by P. V. Somov, whose co-workers were N. K. Grzhebina, V. P. Dzhaneladova, V. P. Romanova, Ye. M. Tsvetkova and others. The impetus for the creation of the department was constituted by two epidemic outbreaks: one was of arthropod-borne type, observed in the summer of 1933 in the Don delta, and the second was of a mixed type (agricultural, domestic and water-borne) in the winter of 1933-1934 on the territory of Rostovskaya (at that time Azovo-Chernomorskiy Kray) and partly in Stalingradskaya Oblasts. In the study of the latter outbreak the role of the house mouse as a source of infection was shown for the first time (A. A. Miller, B. N. Stradomskiy, P. V. Somov and

others). On the basis of the study of the clinical characteristics of disease in people during this outbreak made by workers of the chair of infectious diseases of the Rostov-na-Donu Medical Institute headed by its director B. N. Stradomskiy, anginal-bubonic (B. N. Stradomskiy), abdominal (G. P. Rudnev, Ye. D. Polumordvinova) and pneumonic (G. P. Rudnev) forms of tularemia were distinguished and described. G. P. Rudnev worked out a more complete clinical classification of tularemia than American authors had. B. N. Stradomskiy and I. S. Tinker suggested the water route of infection of people through the consumption of water, which was then proved by P. V. Somov in cooperation with A. A. Vol'ferts and Ye. I. Novikova after isolating cultures of the pathogen from a well contaminated by rodents. In the world literature the role of the water factor in the epidemiology of tularemia had not been noted prior to that. In the summer of 1937 an arthropod-borne outbreak of tularemia recurred in the Don delta, and in the study of it it was possible for the first time to isolate (by a biological test on guinea pigs) cultures of the tularemia microbe from two species of horseflies (P. V. Somov and coauthors, 1940). Subsequently, blood-sucking diptera spontaneously infected with the tularemia pathogen were found in other natural foci by Ye. I. Novikova, V. G. Pilipenko, V. P. Bozhenko and others. Aside from epidemiological studies during the outbreaks mentioned above, a series of investigations on tularemia was made by workers of the Rostov laboratory under the supervision of P. V. Somov subsequently dealing with problems of epizootology, immunology, serotherapy and disinfection.

In 1934, tularemia was found in the Ukraine (Cherkasskiy Rayon). The first cases of disease among people (infections in the water rat and hamster industry as well as in the hare industry) were described by S. N. Ruchkovskiy and coauthors (1935). Beginning with 1935 the Tomsk Institute of Epidemiology and Microbiology began the study of tularemia in West Siberia; S. P. Karpov headed this work. Interest was shown in tularemia because of the finding of cases among people occurring as the result of consumption of infected water from a running brook (S. P. Karpov and N. I. Antonov, 1936) in the summer of 1935 in what was formerly Tayginskiy Rayon of Zapadno-Sibirskiy Kray (now Yashkinskiy Rayon of Kemerovskaya Oblast). This focus was then studied in detail by A. A. Selezneva, and tularemia infection was found in various water-dwelling animals living in brooks infected with tularemia bacteria. In its characteristics this focus belongs to the foothill-brook type (see above).

S. P. Karpov and his co-workers V. M. Popov, A. A. Selezneva, A. F. Komarova, A. G. Slinkina and V. I. Seredina made a series of important studies on the epidemiology, epizootology, diagnosis



**Professor B. N. Stradomskiy**

and prophylaxis of tularemia and contributed much to acquainting medical workers of Siberia with this infection and methods of controlling it.

Beginning with 1938 the Stavropol' Plague Station (now the Scientific Research Plague Institute of the Caucasus and Transcaucasus) began the study of tularemia. In 1940, on the territory of Stavropol'skiy Kray a large autumn-winter outbreak of tularemia occurred, associated with an epizootic among mouse-like rodents. Study of this outbreak made by V. N. Ter-Vartanov, I. G. Ioff, M. P. Pokrovskaya, M. A. Miroshnichenko, I. N. Mayskiy and others added a number of new facts to the epidemiology and epizootology of this infectious disease.

In 1934, data on the study of tularemia in the USSR were reported by L. M. Khatenever, G. Ya. Sinay, A. A. Miller, G. P. Rudnev, D. A. Golov, N. G. Olsuf'yev and others to the All-Russian Conference of Microbiologists and Epidemiologists, at which the problem of tularemia was posed as one of the subjects on the program. At this Conference the medical public heard and discussed for the first time

the initial results of the study of tularemia in the USSR. Specifically, a new classification of clinical forms of the disease was proposed which had been worked out by Soviet authors. In the report data were presented on the epidemiology, epizootology, clinical aspects and diagnosis of tularemia, including the first data on the distinction of a pneumonic form of this disease (G. P. Rudnev) and the extensive application of tularin to people with the aim of diagnosis (L. M. Khatenever and others).

At the beginning of 1938 (1-3 February) at the VIEM the first All-Union Conference on Tularemia was convoked, at which P. F. Zdrodovskiy, A. A. Vol'ferts, N. A. Popov, S. P. Karpov, P. V. Somov, N. G. Olsuf'yev, B. V. Voskresenskiy, P. A. Vershilova and others participated. At the Conference the main results of the study of this disease and of its control in the country were summarized, and further problems were outlined. It was decided to recommend that the Narkomsdrav SSSR organize tularemia stations and departments, by analogy with the malaria and brucellosis systems, entrusting the problems of an organizational-scientific center to the tularemia laboratory of the VIEM. The need was also pointed out for expanding the teaching on tularemia in medical colleges and institutes for the advanced training of physicians as well as publication of appropriate literature and the introduction of compulsory registration of cases of tularemia by public health organs.

In 1938 the first tularemia stations in the USSR were organized -- Fodol'sk (later Mikhnevo) and Zaraysk in Moskovskaya Oblast; Mikhaylov, in Ryazanskaya Oblast; Plavsk, in Tul'skaya Oblast; and Borisogleb, in Voronezhskaya Oblast.

The impetus for organization of them, aside from the resolution of the Conference mentioned above, was a large outbreak of tularemia of agricultural origin observed in January-April 1938 and embracing a number of regions of Moskovskaya, Ryazanskaya, Tul'skaya and Orlovskaya oblasts, as well as water and other outbreaks observed in 1938 in Voronezhskaya Oblast. For the purpose of conducting anti-epidemic measures in foci of these diseases detachments were sent out headed by L. V. Gromashevskiy, G. Ya. Sinay, B. I. Gandel'sman and others. At the end of the outbreak the laboratory centers of these detachments were used for the organization of stations mentioned above, which were given the functions of interrayon institutions.

At the beginning of 1939 (19-23 January) the second All-Union Conference on Tularemia was convoked at the VIEM, which had a greater attendance than the first one. L. M. Khatenever, G. Ya. Sinay, G. P. Rudnev, N. A. Gayskiy, N. A. Popov, B. V. Voskresenskiy, N. G. Olsuf'yev, A. N. Berinskaya, P. A. Vershilova and others participated



**Academician Ye. N. Pavlovskiy**

in it. The Conference was mainly on a summarization of the results of study of the tularemia outbreak in 1938 as well as the results of its control. The main resolutions of the second Conference in the main repeated the resolutions of the first one; specifically it was pointed out once again that there is a need for introducing compulsory registration of cases of tularemia in people. In 1939, data of the study of tularemia in the USSR were reported by G. Ya. Sinay, G. P. Rudnev, N. G. Olsuf'yev, A. N. Berinskaya, B. V. Voskresenskiy, N. A. Gayskiy, P. V. Somov, N. A. Popov, N. K. Gzhechina and others to the All-Union Conference of Microbiologists, Epidemiologists and Specialists on Infectious Diseases. In the reports problems of the

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epidemiology, epizootology, clinical aspects, treatment and prophylaxis of tularemia were discussed, whereby the problem of classification of clinical forms of this infectious disease was subjected to special discussion. In the same year, 1939, at the first conference on parasitological problems all the materials accumulated up to that time on reservoirs of tularemia and vectors of it were analyzed (N. G. Olsuf'yev, 1939) from the viewpoint of natural focalization of the infectious disease in accordance with the general principles of the doctrine of Academician Ye. N. Pavlovskiy which had just been formulated about the natural focalization of arthropod-borne diseases. In 1940 and 1941 special conferences were held on tularemia, but they were convoked by the Narkomzdrav SSSR and were more specialized than the conferences of 1938 and 1939 at the VIEM. The 1941 conference was chiefly on summarizing results of control of the tularemia outbreak observed in Stavropol'skiy Kray in 1940 (see above).

During the period under analysis the first expeditions were organized for the detailed study of natural foci of tularemia. One of them, under the supervision of B. V. Voskresenskiy, worked in 1938 in Mikhnevskiy Rayon of Moskovskaya Oblast. N. V. Nekipelov, Ye. N. Tolstukhina, Ye. M. Tsvetkova, N. N. Uzanov, N. G. Olsuf'yev and others participated in it.

This expedition for the first time established the role of common voles as a mass source of infection of people (the first finding of natural tularemia in these voles in 1936 in the environs of Alma-Ata was made by D. A. Golov), and a number of other reservoirs of the infection in the group of rodents and insectivores was demonstrated. For the first time in the USSR the possibility of tularemia infection of cattle, hogs and horses was established (N. N. Uzonov), and disease in sheep was confirmed. In sheep tularemia was found for the first time by K. A. Dorofeyev and V. I. Gorokhov (1938) in Stalingradskaya Oblast. The studies of the Mikhnevo expedition were continued in 1939-1941 under our supervision, and the role of ixodid ticks was established in prolonged maintenance of the infection during the interepizootic period.

A large comprehensive expedition was organized in 1940 in West Siberia under the direction of L. M. Khatenever. S. P. Karpov, A. F. Komarova, N. A. Popov, V. M. Popov, N. V. Nekipelov, N. G. Olsuf'yev and others participated in its work.

In concluding the presentation of the main works of the period of study of tularemia under analysis, we should make particular note of the very valuable investigations of B. Ya. El'bert and N. A. Gayskiy in the field of pathology and immunology of tularemia and theoretical substantiation of vaccine prophylaxis of this infectious disease.



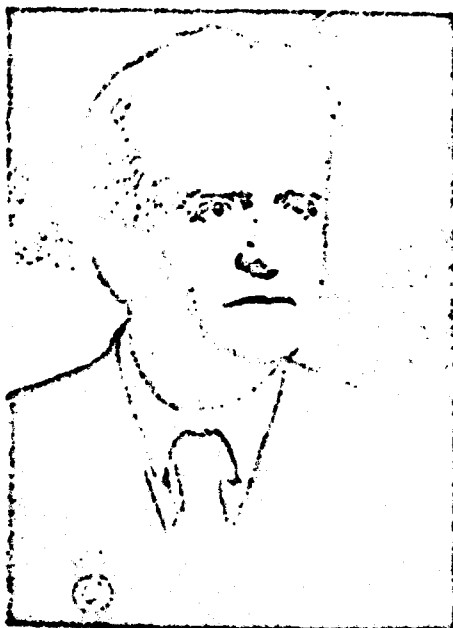
N. A. Gayskiy became acquainted for the first time, for practical purposes, with tularemia during an arthropod-borne outbreak in Balashov (on the Khopra River) in 1931, where he suffered the disease after laboratory infection. The result of combined basic works of B. Ya. El'bert and N. A. Gayskiy (1941) which they made during the period from 1932 to 1936 was a clarification of the main rules and regulations of variation of the tularemia microbe as well as a detailed study of the characteristics of the tularemia infection and mechanisms of immunity in it. Along with the virulent strains the authors had at their disposal a strain with reduced virulence, which in an experiment on animals and in tests on people possessed the properties of a vaccine and caused a distinct immunological reorganization of the body. (This strain, called "Moskva" [Moscow] by the authors, was then lost.) These investigations were stopped for reasons not under the control of the authors and were renewed only several years later.

During the period under analysis valuable investigations were made by P. P. Dvishkov (1930, 1932, 1936), G. V. Vygodchikov and L. M. Khatenever (1932) on the pathology of tularemia, which was further developed in the works of V. G. Molotkova, F. I. Pozhariskiy, L. S. Bobrova, Z. D. Khakhina, V. S. Kolesnik and other authors (see below).

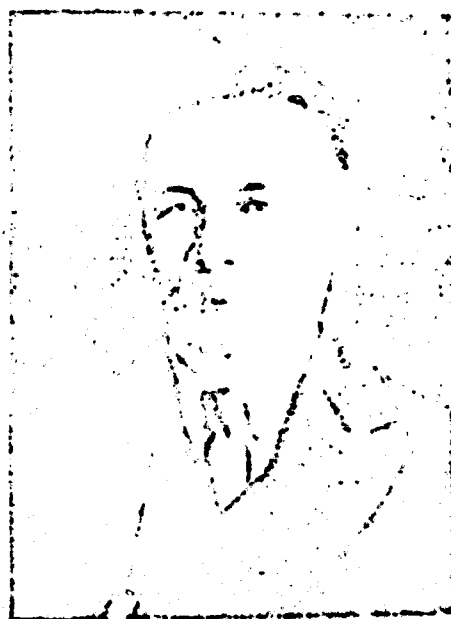
The years of the Second World War (beginning with 1942) and the first post-War years through 1949 can be considered as belonging to the third period of study of tularemia in the USSR. This period is characterized by a considerable expansion (beginning with 1943) of the system of tularemia stations as measures directed against the increased incidence of tularemia during the war years (see below) as well as extensive acquaintance of the medical public of the country with the clinical aspects and diagnosis of this disease. In contrast to the second period the tularemia stations were created almost exclusively as oblast or kray institutions. In their ten years of existence they played a decisive part in the organization of control of tularemia and fully justified themselves. Subsequently, they, as well as other specialized antiepidemic institutions, were combined with the sanitary-epidemiological stations. Because of the work of the tularemia stations there was a marked increase in the knowledge of the clinical aspects and diagnosis of tularemia by the rank and file medical workers.

Major outbreaks of tularemia were observed among the population of different regions of the country both during the period of the Second World War and during the first post-War years (see below).

The increase in the number of registration cases of disease was associated not only with the improvement of diagnosis of tularemia but also with an increase in the epizootic among rodents against the



Professor B. Ya. El'bert



Professor N. A. Gayskiy

background of their mass multiplication. During the War particularly severe epizootics of tularemia were observed on territories which were under the temporary occupation of German invaders, for example, in the Ukraine, in Stalingradskaya, Rostovskaya oblasts and others, as well as in the oblasts of the forest steppe belt of the European portion of the USSR, where agriculture was impaired by the course of the War (Tul'skaya, Voronezhskaya, Ryazanskaya, Orlovskaya, Kurskaya and other oblasts). Tularemia was observed in the battling armies, whereby the German physicians, not being acquainted with tularemia, first diagnosed it as Volhynia fever (A. I. Volkov, 1944, and others). The high incidence of tularemia in the German Army on the Eastern Front was reported by Jusatz (1952), Trautmann (1953) and others.

Soviet scientists G. P. Rudnev, I. I. Yelkin, T. Ye. Boldyrev, K. F. Akinflyev, N. I. Kalabukhov, I. R. Drobinskiy, B. L. Ugryumov, A. F. Bilibin, L. M. Khatenever, I. N. Mayskiy and others under the difficult wartime conditions made an important contribution to the clinical aspects, diagnosis, epidemiology and prophylaxis of wartime tularemia through their studies. Blindage or trench out-

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breaks of tularemia were described (A. I. Volkov, 1944; B. Ye. Nesgovorov and A. A. Chasovnikov, 1944; I. M. Rafalovich, 1943; I. N. Mayskiy, 1944; S. V. Grikurov, 1946; and others), and a study was made of the pathology in tularemia (V. G. Molotkov, 1943; F. I. Pozhariskiy, 1946; A. S. Bobrova, 1949); vaccine therapy was worked out and used clinically (L. M. Khatenever, Ye. M. Tsvetkova, A. F. Bilibin, G. P. Rudnev and others). The results of the control of tularemia under wartime conditions were then generalized on under the general editorship of G. P. Rudnev in a volume of the 32-volume publication Experience of Soviet Medicine During the Second World War 1941-1945. In 1943, at the plenary meeting of the Scientific Medical Council of the Narkomzdrav RSFSR one of the main problems was that of tularemia. In the reports by L. M. Khatenever, I. N. Mayskiy and A. F. Bilibin new data were reflected which had been accumulated during the first years of the Second World War.

Even in the pre-War years the question of vaccine prophylaxis of tularemia arose very pointedly. As was mentioned above, B. Ya. El'bert and N. A. Gayskiy took up this matter particularly successfully. The latter, continuing investigations at the Irkutsk Plague Institute and using methods of attenuation of tularemia bacteria which he had worked out, obtained two strains of reduced virulence at the end of 1941 which, after careful testing on laboratory animals, he used for the preparation of living vaccine. In 1942, he and V. V. Kosmachevskiy made the first successful tests of vaccine on volunteers, and in subsequent years the vaccine was widely tested by N. A. Gayskiy in foci of infectious disease, including Kirovskaya, Ryazanskaya and Voronezhskaya oblasts. The vaccine was used subcutaneously.

In 1945, B. Ya. El'bert and co-workers at the Rostov Plague Institute continued their investigations after showing the possibility of percutaneous application of the vaccine made of the Gayskiy strain, which very much simplified the incorporation of it into practice. (According to the personal report of B. Ya. El'bert, he obtained the first and completely convincing data on percutaneous vaccination against tularemia in 1937 in his work with the "Moskva" strain.) The vaccine was grown in a liquid egg yolk medium proposed by M. S. Droshevskina; this preparation proved to be simple to produce but was inadequately stable on standing, as the result of which it was subsequently replaced by a dry vaccine. The tularemia was first dried by N. A. Gayskiy and Ye. M. Golinevich, but it was finally developed by M. M. Faybich and T. S. Tamarina (1946). The living dry tularemia vaccine of the NIEG [Scientific Research Institute of Epidemiology and Hygiene] proposed by M. M. Faybich is a highly effective preparation and its immunogenic properties are considered a model with

respect to which various institutes are oriented in its preparation. M. M. Faybich and his co-workers also carried out a number of valuable studies on experimental immunology of tularemia and the testing of a living vaccine on people. B. Ya. El'bert and co-workers (1946) in 1945 proved the high degree of effectiveness of percutaneous vaccination when used at the time of a beginning epidemic outbreak. In parallel with the study of vaccination at the Rostov Plague Institute extensive investigations were made on the microbiology, pathogenesis and immunity of tularemia and other problems (I. S. Tinker, M. S. Drozhevskina, Z. D. Khakhina, T. I. Puchkova and others).

In 1946, at the conference on tularemia convoked at the Ministry of Health RSFSR the results of study of this infectious disease in the War years were summarized, including problems of the epidemiology, clinical aspects and prophylaxis. There were many people at the conference; L. M. Khatenever, I. N. Mayskiy, B. Ya. El'bert, N. I. Gayskiy, M. M. Faybich, G. P. Rudnev, A. F. Bilibin, I. G. Akimov, A. A. Maksimov, P. V. Somov, N. G. Olsuf'yev, V. S. Silchenko and others participated in it. The main problem of the conference consisted of the first results of application of living vaccine, presented in the reports of N. A. Gayskiy, B. Ya. El'bert, M. M. Faybich and others. The extensive material accumulated during the entire preceding period of study of tularemia afforded the possibility to a number of investigators of speaking at the conference and then publishing the most complete classification of types of epidemic outbreaks (L. V. Gromashevskiy, I. N. Mayskiy, I. I. Yelkin and others), the types of natural foci (A. A. Maksimov, N. G. Olsuf'yev, later, V. P. Bozhenko, S. P. Karpov and others), and clinical forms of disease (G. P. Rudnev, A. F. Bilibin, A. N. Berinskaya and others).

Along with the Irkutsk and Rostov plague institutes scientific research work on tularemia was conducted at the end of the War and during the post-War years in Moscow at the IEM imeni Gamaleya, at the Stavropol' Plague Station (later reorganized into an institute), at the Moscow Observation Station, Moscow and Tomsk institutes of epidemiology and microbiology, at the chairs of certain colleges (TsIU [Central Institute for Advanced Training of Physicians], Smolensk Medical Institute and others) as well as at various tularemia stations (Voronezh, Tula, Moscow, Stalingrad, Smolensk, Rostov-na-Donu, Mikhnevo, Plavsk and others). In these institutions detailed studies were made in the field of microbiology, immunology, epidemiology, epizootology, zoology, parasitology and prophylaxis of tularemia.

Aside from the works of N. I. Gayskiy and coauthors and B. Ya. El'bert and coauthors mentioned above on immunology and vaccine prophylaxis of tularemia mention should also be made of investiga-

tions of I. N. Mayskiy, V. A. Yudenich, I. S. Tinker and A. V. Mashkov, which they generalized on in doctoral dissertations. The work of O. S. Yemel'yanova on microbiology was completed by the publication of a separate monograph. Of the work done at the Tularemia Station we should like to mention studies on the effectiveness of vaccination by V. S. Sil'chenko (Voronezh) and N. A. Kazberyuk (Ryazan'), generalized on in candidates' dissertations. In the field of veterinary medicine K. A. Dorofeyev (1951) generalized on data on tularemia in agricultural animals, but the large number of distortions and errors in the references made by Dorofeyev in the book which he published, Tularemia in Animals, deprives us of the opportunity of using it for reference purposes.

In the period under analysis a comprehensive study was made of the natural foci of tularemia with the aim of substantiating methods of sanitization of them. Such studies were made by the "Mikrob" Institute in 1943-1945 in the Volga delta (V. G. Pilipenko, G. A. Kondrashkin and others), the IEM imeni Gamaleya in 1946, 1949 in Mikhnevskiy Rayon and adjacent rayons in the south of Moskovskaya Oblast (N. G. Olsuf'yev, N. P. Naumov, I. N. Mayskiy, V. V. Kucheruk, T. N. Dunayeva and others), the Rostov Plague Institute in the Don delta (V. P. Romanova, V. P. Bozhenko, M. G. Yakovlev and others), the Tomsk Institute of Epidemiology and Microbiology in West Siberia (S. P. Karpov, A. A. Selezneva, V. M. Popov). Many years of experience of work in Mikhnevskiy Rayon showed the promise of sanitization of various natural foci of tularemia and were made the basis of the compilation of a number of instructions on tularemia by a group of authors which have been included in the collection of the Ministry of Health USSR, Tularemia. Organizational-Methodological Material.

In places during this period work was done successfully on regional epidemiology, which made it possible to evaluate properly the system of prophylactic measures being taken (G. P. Slavin, A. F. Komarova, Yu. A. Myasnikov and others). Of the group of valuable generalizations of a zoological-epizootological nature made during this period note should be made of the book by A. N. Formozova (1947) on the ecology of mouse-like rodents -- reservoirs of tularemia. At the end of 1949 the Ministry of Health USSR promulgated an order that the tularemia measures be intensified in the country. The basis for this was the large tularemia outbreak in the Ukraine and in Moldavia in 1948-1949 as well as an increase in the morbidity rate in other areas. Provision was made for intensification and regulation of rodent control, including work done for reducing the census of the water rat, and provision was made for intensification of this industry by means of

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increasing the prices to be paid for procuring water rat pelts. Public health organs were advised to expand the system of tularemia stations and intensify inoculations.

This order played an important part in the proper organization of measures and reduction of the incidence of tularemia in the country. To a certain degree it defined the boundary between the periods of study of tularemia analyzed above and subsequent ones. Correspondingly, the fourth or modern period can be regarded as beginning with 1950 and being in existence now. It is characterized by the maximum number of tularemia institutions in the country (reorganized in 1955 into departments of particularly dangerous infectious diseases of the oblast and kray sanitary-epidemiological stations) and by the most complete development of their practical activity as well as by planned mass coverage of the population by inoculations and the taking of other prophylactic measures in regions of the country unfavorable with respect to tularemia. Also, the further mastery of diagnostic and prophylactic measures in tularemia by the medical public has taken place, although in various places the acquaintance with this infectious disease and control of it are still inadequate, which has led to allowing the existence of the disease or incomplete detection of it.

Scientific research work on tularemia during the period under analysis has been conducted in approximately the same institutions as during the previous period, mainly along the same lines. There has been a notable intensification of research work at the tularemia stations (Voronezh, Tula, Stalingrad, Omsk, Pyatigorsk and others). As a summary the experience of study of the effectiveness of inoculations against tularemia was generalized on in a separate collection (N. G. Olsuf'yev, V. S. Sil'chenko, M. F. Shmuter, Yu. A. Myasnikov and others). In an experiment (Ye. M. Tsvetkova, 1951, 1953; V. D. Olli, 1951; Ye. V. Vlasova and coauthors, 1952 and others) and then clinically (G. P. Uglovoy, 1952; R. A. Savel'yeva and G. P. Uglovoy, 1953) the data of foreign authors were confirmed, to the effect that streptomycin is highly effective therapeutically in tularemia. Later, experimentally the lack of promise of using biomycin [aureomycin], terramycin and other antibiotics for tularemia was also clarified (Ye. M. Tsvetkova, 1957; S. A. Tsareva, 1959; Ye. M. Tsvetkova, 1959). Major investigations were made on the variation of tularemia bacteria (O. S. Yemel'yanova). In the study of the antigenic structure a capsular antigen (Vi) was found in the virulent cell with which the virulent and immunogenic factors were associated (N. G. Olsuf'yev and O. S. Yemel'yanova, 1957). Considerable chemical differences were established between the antigenic substances of virulent and avirulent tularemia bacteria (G. K. Shipitsina and O. S. Yemel'yanova, 1956). The

valuable properties of the vaccine strain Gayskiy 15 were restored; this had lost its activity somewhat in recent years as the result of prolonged standing in a museum. A new vaccine strain, 155, was obtained (O. S. Yemel'yanova, 1957). A study was made of the epizootological and epidemiological characteristics of the natural foci of tularemia in the Volga-Akhtubinsk River Valley (N. G. Olsuf'yev, V. V. Kucheruk, V. P. Borodin, N. I. Makarov, V. G. Petrov, Ye. I. Selyanin and others), the Altay foothills (N. G. Olsuf'yev, V. V. Kucheruk, V. G. Petrov, G. P. Uglovoy and others) and the steppe region of the pre-Caucasus (V. P. Bozhenko and S. F. Shevchenko, V. V. Kucheruk, T. N. Dunayeva, V. G. Petrov and others). A study was made of the characteristics of tularemia pathogenesis in more than 50 species of wild animals, and a basis was given to dividing them into three groups according to their relation to the infectious disease (T. N. Dunayeva, N. G. Olsuf'yev). New vectors of tularemia from the group of ticks were demonstrated (V. G. Petrov, Ya. F. Shatas and N. A. Bystrova, Ye. N. Nel'zina; V. P. Bozhenko and S. F. Shevchenko) and methods of controlling them were tried out (V. G. Petrov). Effective methods of control of the water rat were worked out (V. V. Kucheruk and co-authors). Data on regional epidemiology of tularemia were accumulated (for Tula, by Yu. A. Myasnikov; Stalingrad, by V. P. Borodin; Omsk, by O. V. Ravdonikas; Voronezh, V. S. Sil'chenko; Minsk, F. G. Rubanova; Armenia, V. N. Zil'fyan and O. V. Avoasapyan and others).

This is the far from complete list of the basic results of research work done in the period under analysis. We should like to mention that after going from Rostov to Minsk (1949) B. Ya. El'bert continued to be interested in the study of tularemia and particularly in problems of vaccine prophylaxis. Under his supervision two candidate's dissertations were written at the Smolensk Institute of Epidemiology and Microbiology (A. L. Matskevich, 1952, M. M. Kirvel, 1953), and at the Loyev Rayon Sanitary-Epidemiological Station one candidate's dissertation was written (I. L. Martinevskiy, 1954).

At the end of 1949 (31 October-1 November) and then in the middle of 1951 (11-12 June) on the initiative of Ye. A. Pronina conferences were convoked at the Ministry of Health RSFSR for the study of the effectiveness of tularemia inoculations. At the beginning of 1953 (17-19 February) and in the middle of 1955 (11-12 May) at the same Ministry another conference was held on tularemia, but in a topical respect it was of a broader category. The conferences played an important part in summarizing scientific research and practical work which had been done and in defining problems for the future.

Problems of the study of the natural focalization of tular-

emia have been extensively discussed at the scientific session of the IEM imeni Gamaleya convoked in March 1954 in honor of the 70th birthday of Academician Ye. N. Pavlovskiy as well as at the 10th conference on parasitological problems and diseases with natural foci convoked in October 1959 by the Institute of Zoology of the Academy of Sciences USSR and the IEM imeni Gamaleya. A valuable summary of the results of the 30-year study of tularemia in the USSR was made up by V. S. Sil'chenko (1957). He published a thorough outline on the history of vaccination against tularemia (V. S. Sil'chenko, 1955).

We should like to dwell briefly on problems of the distribution of natural foci of this infectious disease on the territory of the USSR and the movement of the tularemia morbidity over the period since it began to be accurately diagnosed, that is, beginning with 1926. As has been mentioned above, during the period 1926-1928 tularemia was found almost simultaneously in several places of the RSFSR including the Volga delta, on the Oka River in what was formerly Ryazanskaya Guberniya, on the Ural River, in what was formerly Orenburgskaya Guberniya, on the Irtysh and Obi within limits of what was formerly Tobol'skiy Okrug and in Yakutiya. After two years, Southeast Kazakhstan was added to the known area of distribution and after the next five-six years, the Ukraine, North Caucasus, south of Moskovskaya Oblast, etc. were added. At the present time, natural foci of tularemia have been demonstrated on the territory of the western boundaries of the country (Kola Peninsula, Karelia, Estonia, Latvia, Kaliningradskaya Oblast, Belorussia, Ukraine, Moldavia) and in the East as far as Yakutsk and Khabarovsk. To the North, foci of tularemia are found in places as far as the Arctic Circle; in the South, the area of distribution includes the Crimea, Transcaucasus, Turkmenia and South Kazakhstan (for more details see Chapter V, page 173).

The following question arises: was the expansion of the boundaries of tularemia during the period under analysis the result of spread of the infection from one oblast to another, or was it simply a matter of detection of foci previously in existence? Jusatz (1952, 1955) believes that between 1920 and 1930 tularemia spread in a wave-form from Siberia to the European part of the USSR and then broke out in countries of Western Europe (for more details see Chapter II, page 48). Jusatz's viewpoint, which he has repeatedly expressed in his articles and, unfortunately, which has already partially penetrated into the literature of other countries, should be considered incorrect and disorienting. It is based on lack of knowledge or ignorance of the factual data of the distribution of tularemia in the USSR and in the countries of Western Europe. As has been mentioned above, descriptions existing in the Russian pre-revolutionary medical literature in...



the 19th and beginning of the 20th centuries leave no doubt of the fact that tularemia was widespread on the territory of Russia long before it was learned how to diagnose it, not only in Siberia but also in the European part of the country. This should be stated also with respect to Western European countries, where tularemia has been known at least since the 17th century (see Chapter II, page 43). All this gives us reason to believe that tularemia in Europe and in the USSR has been widespread since ancient times, and the detection of its foci is not associated with "invasion waves" from one territory to the next but rather with a diffusion of knowledge about this disease among physicians, including methods of accurate diagnosis. Incidentally, we should like to note that the geography of natural foci of tularemia has been studied at present in the USSR inadequately, particularly in Eastern Siberia, in the Far East and in Central Asia. There are also considerable "blank spots" within the limits of the European portion of the USSR which require appropriate checking on the subject of whether tularemia is really absent there. What has been stated does not exclude the possible pulsations of the areas of distribution of various natural foci within the general region of distribution of the infection. Such changes in the boundaries of the foci are well known, but their scale is generally small and at the present time they do not change the basic geography of the tularemia pathogen.

The incidence of tularemia among people has undergone considerable variations by years in the USSR. During the first and second periods of study of tularemia, that is, from 1926 through 1941 it was noted only in a small number of oblasts in connection with the fact that its diagnosis was available to only a limited number of physicians, particularly during the initial period. During these periods, undoubtedly, many cases were missed, although in various years (1933-1934, 1938, 1940) they assumed the nature of large outbreaks. The list of tularemia outbreaks noted during the period from 1926 through 1942 has recently been published by N. V. Nekipelov (1959). We should like to note that compulsory registration of tularemia was introduced over the entire country only in 1941. During the years of the Second World War the number of cases of tularemia increased sharply and continued to be high during the post-War years (Fig 1). The causes of rise in the morbidity during this period have been mentioned above. During the War years and later clear-cut three-year variations in the incidence of tularemia associated with variations in the census of small mouse-like rodents were noted, from which during this period the main mass of infections of people occurred in the European part of the USSR. It should be noted that the true incidence of tularemia during this period was higher than has been established by official

statistics, which has been clarified as the result of special studies in Moskovskaya (N. G. Olsuf'yev, I. N. Mayskiy), Tul'skaya (Yu. A. Myasnikov), Stalingradskaya (V. P. Borodin) and other oblasts (see Chapter VI).

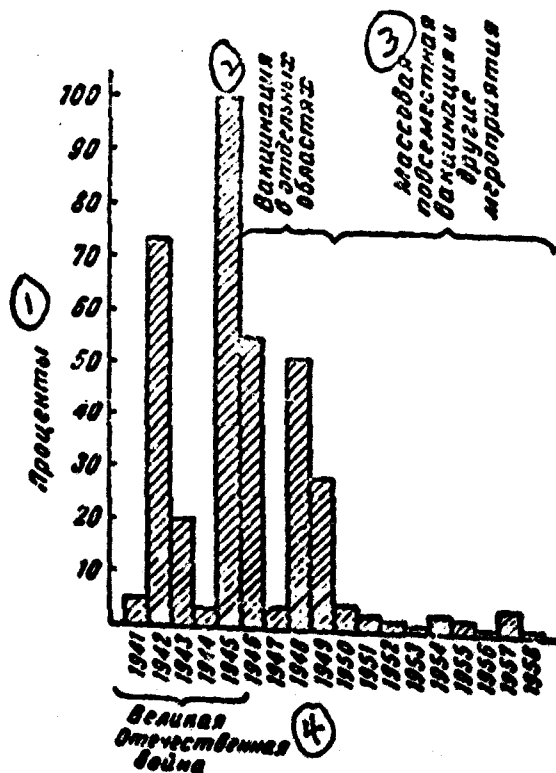


Fig 1. Movement of the Incidence of Tularemia in People in the USSR from 1941 through 1958. The 1945 morbidity rate is taken as 100 per cent. 1. Percentages; 2. Vaccination in various oblasts; 3. Mass general vaccination and other measures; 4. Second World War.

Beginning with 1950, that is, in the last of the periods which we are analyzing, the incidence of tularemia decreased sharply and is remaining at low figures. This reduction is the result of extensive incorporation of vaccination in combination with other measures. Among the latter is the improvement in the quality of tillage of fields and in gathering the harvest, which has reduced the possibility of mass multiplication of small mouse-like rodents. In places, industry has become an important restraining factor on the growth of the water rat census, and this also limits the spread of tularemia epizootics. The control of ticks being conducted in a number of kraya and...

oblastic also contributes to sanitization of natural foci of infectious disease.

### Conclusion

As is seen from this brief historical outline, many scientists have taken up the study of tularemia in the USSR, which is in accordance with the importance of the problem. Even though in the Soviet Union tularemia was found later than, for example, in the United States, Soviet investigators undoubtedly have priority in world science with regard to many divisions of the study of this disease. Specifically, successful vaccine prophylaxis of tularemia has been developed entirely by Soviet scientists. In the USSR at the present time 1300 scientific works have been published on tularemia; 11 doctoral and about 60 candidates' dissertations have been produced and defended.

"The Soviet Union should and will be the first country in the world to eliminate this infectious disease on its territory. With the possibilities which we have this problem is within our power, and confidence in victory will assist us in working it out successfully" -- this is what a noted enthusiast in the study of this infectious disease and one of its discoverers in the Soviet Union, Alevtina Aleksandrovna Vol'ferts, wrote in 1935 in her review on tularemia.

During the 25 years which have passed since that time medical workers of the Soviet Union, combining the achievements of science and practice, have attained great results in the field of the control of tularemia, markedly reducing its incidence among people. It is to be hoped that in the next few years they will make even greater progress.

## Chapter IV

### Experimental Tularemia in Laboratory Animals

#### General Comments

Guinea pigs, white mice and recently white rats have been used extensively in laboratory experiments as models for solving various problems of the pathogenesis, immunity, therapy, epizootology and other branches of the study of tularemia. White mice and partly guinea pigs are used all the time for biological testing with the aim of detecting the pathogens of this infectious disease in objects under investigation (the organs of wild animals, blood-sucking arthropods, pathological material from sick people, etc.). Finally, rabbits are often used for immunological experiments as well as for obtaining agglutinating sera. All this justifies putting the data on experimental tularemia in laboratory animals into a special chapter, particularly since many problems of a general and specific nature being presented in the subsequent chapters can be discussed satisfactorily only with consideration of the results of experimental studies.

Abroad, McCoy, Francis and Downs, and in the USSR G. Ya. Sinay, L. M. Khatenever, B. Ya. El'bert and N. A. Gayskiy, I. S. Tinker and M. S. Drozhevskina, A. V. Mashkov and A. F. Tarnenko and others have taken up the comparative study of experimental tularemia in laboratory animals. The data of these investigations have played an important part in evaluating the main characteristics of tularemia in various laboratory animals.

It is important to emphasize in principle that for the purpose of elucidating the rules and regulations inherent in the natural infectious disease only epidemic or focal strains which are complete with respect to virulence and which have not been involved by attenuation during their preservation under laboratory conditions should be used experimentally for the reproduction of tularemia. Another important condition is accurate dosaging of the pathogen and use of relatively small quantities of bacteria under standard conditions or conditions close to the natural in administering them for the infection. The use of increased doses distorts the development of the infectious process and is permissible only in special experiments when there is a need for such doses. Finally, appropriate attention should be paid to the quality of experimental animals as well as to the conditions under which they are kept (housing, feeding, surrounding temperature, etc.).

Experiments published satisfy the requirements.

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listed, which limits the possibility of using them. In writing this Chapter we used as a basis chiefly materials obtained in recent years by us and co-workers in our laboratory by way of a fundamental revision of the problem. These investigations mainly were accomplished with the use of strains 503 or 9 which were passaged through animals for the purpose of preserving their original virulence; the first strain, through guinea pigs; the second, through white mice (for more details about these strains see Chapter III). In the experiments a culture was used which was isolated from a routine passage.

In the first stages of study of tularemia in laboratory animals by different authors attention was directed to the fact that this infection in various species of animals proceeds differently. For example, white mice and guinea pigs, when injected with minimum doses of the pathogen, become acutely sick and die. On the other hand, white rats and rabbits show a definite resistance to tularemia, and a fatal outcome of the disease in them is observed only after the injection of massive doses of the pathogen. Various authors have considered these differences different degrees of "susceptibility" or "resistance" of these animals to infectious disease. The investigations discussed below showed, however, that such animals as white mice and white rats can be infected with practically the same minimum doses of the pathogen although the subsequent clinical course of the disease and its outcome in these species of animals are absolutely different. Hence, the need arose, as applied to problems of studying tularemia, of clarifying the concept of susceptibility to infection, considering "infectibility" a synonym of it, whereas the reactivity of the animal to infection was considered better designated by the term "infectious sensitivity" (N. G. Olsuf'yev and coauthors, 1950). Essentially, susceptibility and infectious sensitivity of the animal are projections of the properties of the pathogen in the organism -- of its invasive power and pathogenicity. Conversely: invasive power and pathogenicity do not represent any abstract properties of the microbe but rather are expressed in a specific relation of the macroorganism, its susceptibility and infectious sensitivity to it. Correspondingly, the immunizatory properties of the microbe find their reflection in the immunizability (P. F. Zdrovovskiy) or immunogenic reactivity (Sh. D. Moshkovskiy) of the macroorganism. The very evident interdependency of these properties of the macro- and microorganisms can be represented in the following graphic schema (Sh. D. Moshkovskiy (1948) proposed a similar schema but with somewhat different terminology: invasiveness --- resistance; noxiousness --- resistance; immunogenicity --- immunogenic reactivity):

## Properties of the Microbe

Properties of the Animal Organ-  
ism

Invasiveness



Susceptibility

Pathogenicity



Infectious Sensitivity

Immunogenicity



Immunizability

(We have in mind the ability of the organism to be immunized by antigens of the microbe. This property of the macroorganism is also called "immunologic reactivity", but because various authors ascribe different significance to this term we are refraining from using it in this case).

The degree of susceptibility to infection of one type of animal or another is determined by the lowest infective dose of the pathogen (MID) for it, without relation to the outcome of the disease. The degree of infectious sensitivity was conveniently characterized by the lowest dose causing death of all the infected animals (MLCD) in tularemia. Naturally, in other infectious diseases the criteria of infectious sensitivity may be different. Finally, the degree of immunizability of the organism may be determined by the lowest dose of the antigenic substances of the pathogen conferring immunity of adequate strength to the infection (with consideration of the weight of the macroorganism).

The characteristics of warm-blooded animals with respect to their susceptibility, infectious sensitivity and immunizability to tularemia were fruitful for the study of problems of epizootology, immunology and other branches of the study of this infection. In animals with different degrees of infectious sensitivity differences in the nature of tularemia are manifested not only in the outcome (death in highly sensitive animals and recovery in the slightly sensitive species) but also in the intensity of multiplication of bacteria in the organs at different stages of development of the infection.

For the purpose of studying the quantitative aspect of bacteriology of the infection recently various authors (Larson, 1945; Downs and coauthors, 1949; A. V. Mashkov, 1952; as well as our laboratory (V. G. Petrov and N. G. Olsuf'yev, 1953); T. N. Dunayeva, R. A. Savel'yeva) have been using the "titration" method of a suspension of organs and tissues of investigated animals on white mice. It consists of the fact that pieces of the organ or tissues being investigated are carefully ground up in a mortar after being put into suspension, physiological saline solution is added in a proportion of 1:5, further dilutions are made of 10, 100, 1,000, etc. times and these latter are injected subcutaneously in doses of 0.5 cc into white mice, using

three mice for each dilution (Fig 10). The final dilution causing death of all three experimental mice from tularemia indicates the number of complete lethal mouse doses (that is, the MLCD) in one gram of the organ being investigated. In the conversion for the number of bacteria one uses as a basis the fact that in the case of mice one lowest complete lethal dose (MLCD) of the virulent strain corresponds to one microbe by the GKI [State Scientific Control Institute] bacterial standard. This method proved to be simple in its application and quite accurate.

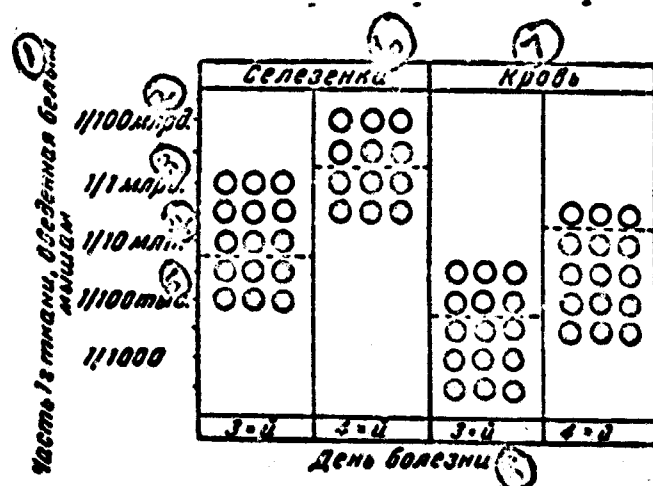


Fig 10. Experiment in Titration of the Spleen and Blood of a Vole with Tularemia on White Mice with the Aim of Determining the Number of Tularemia Bacteria in One Gram of Tissue. The black circles show mice which died of tularemia; the white circles, the surviving mice. Each circle corresponds to one animal. The broken horizontal lines represent the maximum dilutions of the suspension obtained from calculation of the number of bacteria per gram of tissue. 1. Fraction of one gram of tissue injected into white mice; 2. 1/100,000,000,000th; 3. 1/1,000,000,000th; 4. 1/10,000,000th; 5. 1/100,000th; 6. Spleen; 7. Blood; 8. Day of disease.

For the purpose of determining the number of tularemia bacteria in animal organs the method of culturing dilutions of the suspensions on dishes containing glucose-cystine-blood agar was also used (Downs and coauthors, 1947; Hopla, 1953). According to our data, the results obtained from the use of cultures of suspensions on petri dishes containing blood media are almost the same as the results of titration on white mice (T. N. Dunayeva).

In the present Chapter only data on the relations occurring in the bodies of laboratory animals after the effect of virulent strains

of tularemia bacteria on it have been included. The corresponding relations, occurring from the injection of attenuated (vaccine) strains of tularemia bacteria into the body of a warm-blooded animal, will be analyzed in Chapter IX. Presenting various data from the literature on experimental tularemia in laboratory animals we, with very minor exceptions, shall overlook the American data because of the considerably greater virulence of American strains by comparison with Soviet strains and, therefore, the incomparability of a number of experiments. American strains are also virulent for domestic rabbits, for white mice and guinea pigs and are definitely more virulent than Soviet strains for white rats (see Chapter III).

### Experimental Tularemia in Guinea Pigs

A high degree of susceptibility and infectious sensitivity to tularemia, availability for clinical observations during the period of disease, the demonstrativeness of pathological changes at autopsy, the convenience of laboratory maintenance, etc., characterize the guinea pig as an exceptionally convenient model for studying tularemia experimentally. In guinea pigs tularemia occurs like an acute infection ending, as a rule, in the death of the animal. The guinea pig can be infected by any method of the group of those usually used in laboratory practice. However, with different methods of administration of the tularemia bacteria into the guinea pigs the least infective dose differs, and the development of the infectious process has its own characteristic features. By comparison with other species of laboratory animals experimental tularemia in guinea pigs has been most fully studied at present.

The Characteristics of Tularemia after Subcutaneous and Intradermal Infection. The Susceptibility and Infectious Sensitivity. In the case of subcutaneous infection with strains which have their complete virulence the least complete infective (MID) and ~~minimum~~ full lethal dose (MLCD) coincide, amounting to one microbe ( $10^{-9}$ ) according to the GKI optical standard. Death of the guinea pigs from this dose usually occurs eight-11 days after infection, rarely later. A dose of 0.1 microbe ( $10^{-10}$ ) is responsible for the infection and death of guinea pigs only in a few cases and usually in the period from the 11th to the 13th day. Guinea pigs are almost as susceptible to intradermal infection as to subcutaneous. From a dose of one microbe death of guinea pigs is usually observed after 10-13 days. However, after this dose sometimes the guinea pigs survive and they prove to be uninfected, which apparently depends on the poorer survival of bacteria by this method.



Rare cases are known where guinea pigs recovered from the intradermal injection of one or even 10 microbes of a fully virulent strain. An analysis of these cases shows that the animals subjected to infection had an altered immunological state either because of a preliminary injection of tulaxin (for the purpose of checking the skin allergic reactivity) or because of an intercurrent disease which increased the natural defensive forces of the body. With increase in the infective dose the survival time of the guinea pig is shortened, which is shown even after doses of one, 10 and 100 microbes, if we calculate the average figures, but differences are particularly great after the use of larger doses, for example, after subcutaneous or intradermal injection of 100,000,000 or 1,000,000,000 microbes the guinea pigs die after three or even two-and-a-half days (Table 10).

Table 10

Survival Time of Guinea Pigs from Tularemia after Subcutaneous and Intradermal Injections of Different Doses of a Virulent Strain (No 503) of Tularemia Bacteria (after T. N. Dunayeva and O.S.Yemel'yanova)

Доза заражения (микробных клеток)	Подкожно				Внутрикожно			
	зара- жено сви- нок	умро от туля- ремии	средний срок ги- бели (сутки)	колебания срока гибели (сутки)	зара- жено сви- нок	умро от туля- ремии	средний срок ги- бели (сутки)	колебания срока гибели (сутки)
1	10	10	9,8	8-11	12	10	11,6	10-13
10	10	10	8,6	8-11	12	12	10,0	9-13
100	20	20	8,3	6-12	10	10	9,9	8-13
100 млн.	5	5	4,4	3-5,5	3	3	4,7	3,5-5,5
1 млрд.	5	5	3	2,5-3,5	3	3	3,5	2,5-4,5
10 млрд.	4	4	2	2	(10) опыты не ставились			

1. Infective dose (microbes); 2. Subcutaneously; 3. Intradermally; 4. Guinea pigs infected; 5. Died of tularemia; 6. Average survival time (days); 7. Variations in the time of death (days); 8. 100,000,000; 9. 1,000,000,000; 10. Experiments not performed.

The initial stages of attenuation of the strains when kept in a museum quite rapidly have an influence on the virulence of the bacteria for guinea pigs and a dose of one microbe and then 10 microbes no longer produces a fatal result in the guinea pigs (see Chapter III).

**Clinical Aspect.** Elevation of the body temperature in guinea pigs serves as one of the main clinical symptoms characterizing development of the disease. Time of the occurrence of the temperature reaction is closely related to the size of the infective dose and the method of infection. In our experiments, after intradermal infection with a dose of one microbe, the temperature elevation (above  $39.5^{\circ}$ ) in guinea pigs was noted after four or five days, and after infection with a dose of 10 microbes, after three or three-and-a-half days. With increase of the dose the temperature reaction appears more rapidly. Thus, for example, after intradermal or subcutaneous injection of a dose of 100,000,000 microbes the temperature in the guinea pigs increases after five-seven hours, and in some cases, after three-four hours; with a dose of 1,000,000,000 microbes the temperature elevation occurred after three-four hours and in a few cases four-five hours or after two hours, and with increase of the dose to 10,000,000,000 microbes the guinea pigs began to have fever after three hours; at this time, the body temperature was higher than  $40^{\circ}$  in the majority of animals.

At the site of intradermal injection of the bacteria the occurrence of a limited area of redness and slight edema of the skin are characteristic. With minimum infective doses (one-10 microbes) these changes can appear one-two days before the rise in the body temperature. A local focus of inflammation, gradually enlarging, is converted into a readily noticeable papule and then into a pustule, in the center of which tissue necrotization occurs, and in long drawn-out cases of the disease -- ulceration. In the case of subcutaneous infection in the area of injection of the bacterial suspension edema of the tissues appears which increases with the development of the disease and is readily found on palpation. In the region of the injection site (intradermal or subcutaneous) an enlargement of the lymph nodes is noted to the size of a pea, bean, or even walnut, as well as edema of the surrounding tissue. The bubo which develops here is readily found on palpation, whereby the sick guinea pig reacts markedly when it is touched, which indicates the tenderness of the bubo.

The temperature usually remains high and is relatively constant for several days, frequently reaches  $41^{\circ}$  at the height of the fever; then, it falls by crisis or lysis, and its fall below normal temperature indicates the onset of the agonal period. At this time the guinea pigs lose considerable weight, become sluggish, refuse food, their fur is ruffled, their eyes are closed, and their lids are inflamed. Gravid guinea pigs can abort at this time. The agonal period usually begins the day before death. In cases where the guinea pigs recover, which happens when they are infected with minimum doses of museum

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(partially attenuated) strains, the recovery drags on to a month or more. Usually, the regional lymphadenitis is concluded with a purulent destruction of the most affected lymph nodes and the excretion of pus through the fistula formed in the skin, after which a slow resorption of the bubo occurs with cicatrization of the injured tissue. The temperature comes to normal only gradually; recovery of the weight also proceeds slowly.

Pathology. On autopsy of the guinea pigs which died of tularemia typical changes are found which consist of a lymphadenitis and periadenitis, focal necrotization of the splenic tissue, of the liver, regional lymph nodes, partly of the lungs and other organs against the background of a general picture of septicemia. Pathological changes in tularemia in guinea pigs are characterized by marked vascular disorders as well as signs of exudation and proliferation (P. P. Dvishkov, 1930; S. V. Kagramanov, 1943; A. V. Mashkov, 1952). Proliferative changes have the nature of specific granulomas which have much in common with tubercles from tuberculosis. The granulomas consist of epithelioid and lymphoid cells with an admixture of polymorphonuclear leucocytes and histiocytes (Fig 11, A). Beginning with the center, the granulomas become necrotic, and in the case of a far-advanced focus extensive areas of necrosis are formed in the focus of inflammation (Fig 11, B).

With intradermal infection, edema of the tissues with a focus of necrosis in the center surrounded by an area of hyperemia is noted in the affected area of skin macroscopically, whereas microscopically exudative inflammation and an active granulomatous process are found accompanied by extensive areas of necrosis. The regional lymph node (or group of nodes) is markedly enlarged, thickened, and on section its central part is necrotic, frequently in a state of caseous degeneration; if death occurs in later periods the lymph node is converted into pus.

In the case of the subcutaneous infection of the hind leg usually practiced, a dense infiltrate with foci of necrosis and hemorrhages adherent to markedly enlarged and pathological lymph nodes is found in the inguinal area on the side on which the material was injected. The retroperitoneal (lumbar) lymph node regional to the site of infection is also markedly enlarged (to the size of a pea), thickened and necrotic to various degrees. Various degrees of enlargement of distant lymph nodes are also noted, usually more pronounced on the side of the body on which the infection was produced. In these cases the lymph nodes are thickened, congested, but usually without any macroscopically noticeable tissue necrosis. Histologically, in these nodes hyperplasia of the tissue is found, usually without any structural dis-

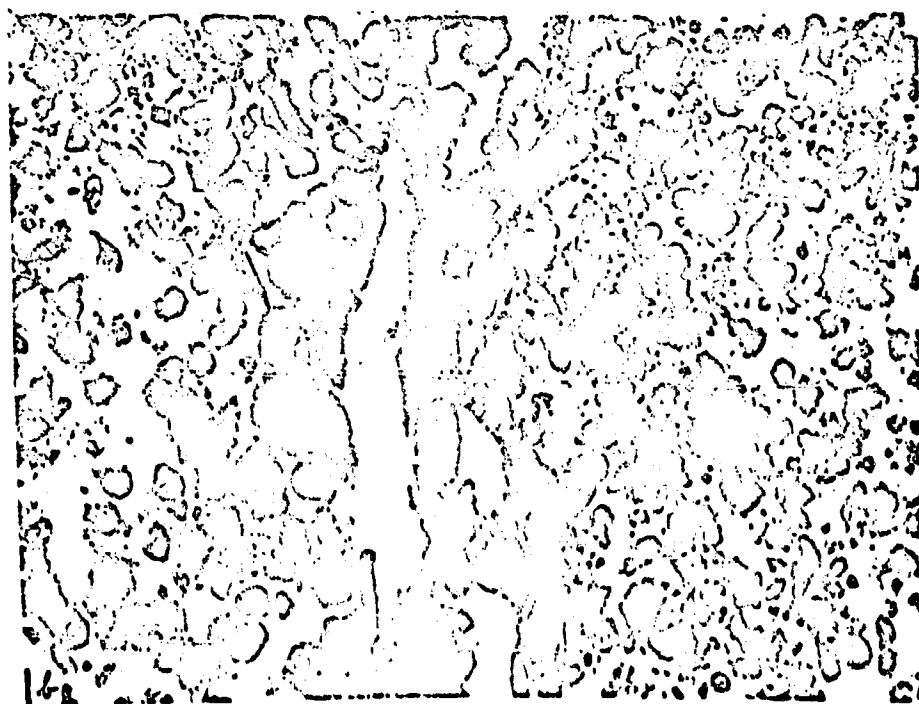
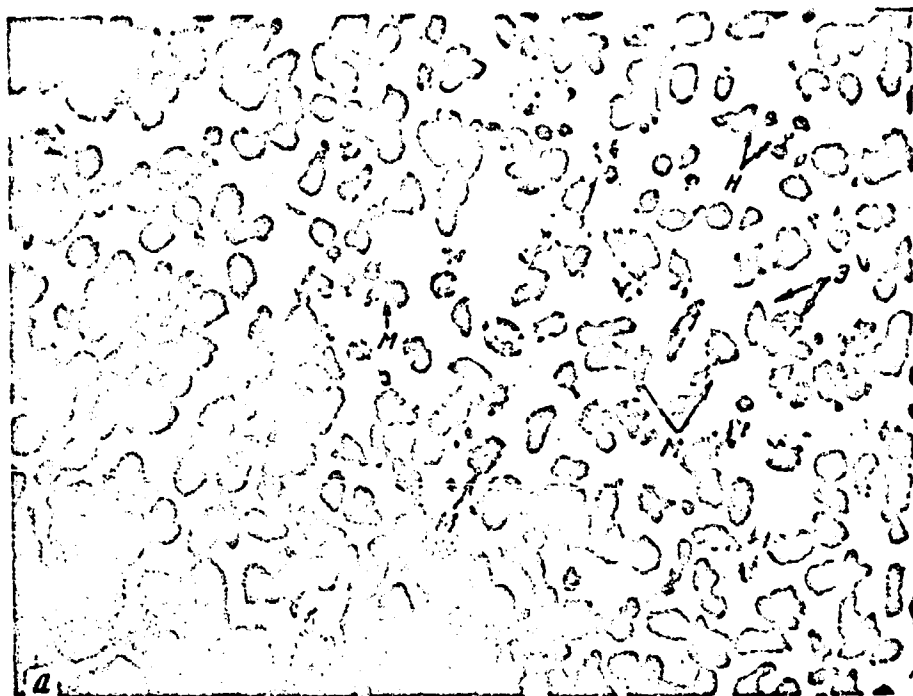


Fig 11. Histopathologic Changes of Tissues in the Guinea Pig in Tularemia. 600x magnification. Photo from preparations by A. P. Gindin. a. Center of fresh tularemia granuloma in lymph node; macrophages (M), epithelioid cells (E) and neutrophilic leucocytes (H) are seen; b. Tularemia granuloma in the stage of necrosis in the splenic tissue; in the center there is an intact blood vessel with proliferating adventitial cells (A).

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order. The blood vessels of the subcutaneous tissue, including the smallest, are usually congested. The spleen is enlarged several times, thickened, raspberry- or cherry-colored, frequently tears easily under the forceps, and is solidly permeated by numerous, well-outlined whitish or grayish necrotic nodules usually the size of a millet grain or semolina. The liver is markedly enlarged, thickened, usually has a clay color, is also permeated by numerous necrotic nodules, and in various cases these become confluent into quite large areas of necrosis of irregular shape. The suprarenal glands are markedly enlarged, hyperemic to varying degrees or contain hemorrhages. The lungs frequently are hyperemic to varying degrees with isolated necrotic nodules measuring the size of a millet grain or smaller. The small intestinal walls frequently are thinned out for different distances; an orange-yellow or bloody content is seen through them. In a number of cases the kidneys are anemic. Congestion of the blood vessels of the mesentery and brain is noted. In case of short lifespans of the guinea pigs (two-four days) caused by injection of massive doses of the pathogen the spleen and the liver are comparatively little enlarged, without noticeable macroscopic necrotic nodules but are markedly thickened; the regional lymph nodes are not much enlarged either but are partially necrotic. The marked hyperemia of blood vessels of the subcutaneous tissue and suprarenal glands attracts attention. In the case of a chronic course of the disease the pathological changes and, particularly, necrotization of affected tissues is maximally expressed. For example, the spleen can be enlarged four-five or more times; necrotic nodules in it reach the size of a buckwheat grain, etc.

Pathogenesis and Microbiological Diagnosis. I. S. Tinker and M. S. Drozhevskina (1948), I. N. Mayskiy (1949, 1953), A. V. Mashkov (1952), A. G. Kratinov and coauthors (1946) and others, and recently workers in our laboratory (T. N. Dunayeva and R. A. Savelyeva) have taken up the study of the pathogenesis of tularemia in guinea pigs chiefly by the subcutaneous method of injecting the bacteria. These studies are of great importance for understanding pathological processes occurring in the body of the person sick with tularemia, despite the dissimilarity of the degree of sensitivity to this infection between the human and guinea pig organism.

The spread of tularemia bacteria in the guinea pig organism as well as the response morphologic changes of tissues are elucidated by means of serial dissections. By means of such a sensitive method as the biological test on white mice it is possible to show even single bacteria in an investigated piece of organ. Cultures on coagulated egg yolk medium and microscopy of smear-impressions from organs also find application, although they are considerably poorer than

the results of the biologic method (see below). The method of titration of investigated organs permits the quite satisfactory determination of the number of bacteria per gram of tissue.

In the guinea pig the infectious process in tularemia, just as in some other diseases with a similar pathogenesis, can be divided into the following phases: I -- adaptation; II -- regional infection; III -- hematogenous dissemination and focal spread of the infection (we have in mind a localized spread of the pathogen through the organs and tissues); IV -- septicemia. The last two phases represent different stages of development of generalized infection. In case of a favorable outcome of the disease (which can be observed after infection with small doses of partially attenuated strains) the phase of hematogenous dissemination (focal spread) is replaced by a phase of extinction of the infection (IVa).

The initial stages of multiplication of tularemia bacteria occur in the tissue at the site of their injection, and here the initial inflammatory changes arise.

T. A. Kalitina and A. P. Gindin found a well-expressed inflammatory reaction in the form of accumulation of polymorphonuclear leukocytes, partial degeneration of them, dilatation and congestion of blood vessels with erythrocytes and the occurrence of histiocyte proliferation around the blood vessels in the dermis and fatty tissue under it at the injection site of a bacterial suspension. At this time the skin at the injection site of the bacteria remained macroscopically unchanged. In subsequent days histopathological changes at the site of bacterial injection rapidly increased and as early as after four days the formation of granulomas was noted from epithelioid and other cells (see T. A. Kalitina, 1953, 1956).

Experiments with intradermal or subcutaneous infection of guinea pigs showed in all evidence that only after the initial stages of multiplication at the site of incorporation are the tularemia bacteria carried to the regional lymph node (or group of closely arranged lymph nodes) along the lymphatics and here they continue to multiply, also producing specific inflammation (I. S. Tinker and M. S. Drozhevskina; Z. D. Khakhina; N. G. Olsuf'yev and T. N. Dunayeva; R. A. Savel'yeva and G. P. Uglovoy and others).

According to our data (N. G. Olsuf'yev and T. N. Dunayeva), after the intradermal injection of a dose of one or 10 microbes of a fully virulent strain into guinea pigs the adaptation phase lasts from two to three days, whereby the bacteria can be found regularly (by the biological test method) in the skin only after 24 hours; prior to this only a few examinations are positive. The phase of regional infection lasts, on the average, about 24 hours, whereby in the case of

infection in the area of the hind leg findings of bacteria are limited to the lymph nodes regional to the site of infection, whereas in the retro-peritoneal lymph nodes bacteria are not found, as a rule, at this time. In total, with these minimum doses of infection mentioned above, the adaptation and regional infection phases last from three to four days, and during this entire period the body temperature remains normal, and weight remains the same in the guinea pigs.

The transition to the phase of hematogenous dissemination, which was established by means of finding bacteria in the spleen, clinically almost coincides with the onset of the fever, preceding it by several hours (Fig 12). In guinea pigs killed on the first day after temperature elevation tularemia bacteria are found regularly in the internal organs and irregularly, in the blood.

From the regional lymph node the bacteria go into the blood stream with the lymph flow and settle out in the organs rich in reticulo-endothelial cells, chiefly in the spleen. Here, the bacteria multiply, also partially penetrate into the blood and by this means spread further throughout the organs and tissues. With the penetration of the bacteria into the blood and then into the parenchymatous organs the second period of the disease begins, which includes the phases of hematogenous dissemination and septicemia and is characterized by the presence of clinically overt, general (fever) and local (inflammation) reactions. During this period, the granulomatous process includes all the organs to which bacteria are carried. In our experiments, after the intradermal injection of one-10 microbes, the febrile period, which includes the phases of hematogenous dissemination and septicemia, lasted six-seven days in guinea pigs; the guinea pigs died on the 10th-11th day after infection.

The phase of hematogenous dissemination (and focal spread of the infection) is characterized by the irregular finding of bacteria in the blood and a relatively moderate seeding of the internal organs and lymphatic apparatus with bacteria.

For example, in the spleen during this period from 1,000 to 100,000 microbes are found per gram of tissue (by the method of titration on mice) and somewhat more are found in the regional lymph node -- up to 1,000,000 per gram of tissue. In the liver, kidneys, bone marrow and lungs bacteria during this period are found less regularly. By cultures on coagulated egg yolk medium bacteria are found chiefly in the spleen and regional lymph node, but growth is late, in the form of separate colonies, appearing on the third-seventh day of standing in an incubator. Comparison of the number of microbes in the guinea pig organs found by titration by the biologic method with the results of cultures from smears of the same organs showed that the

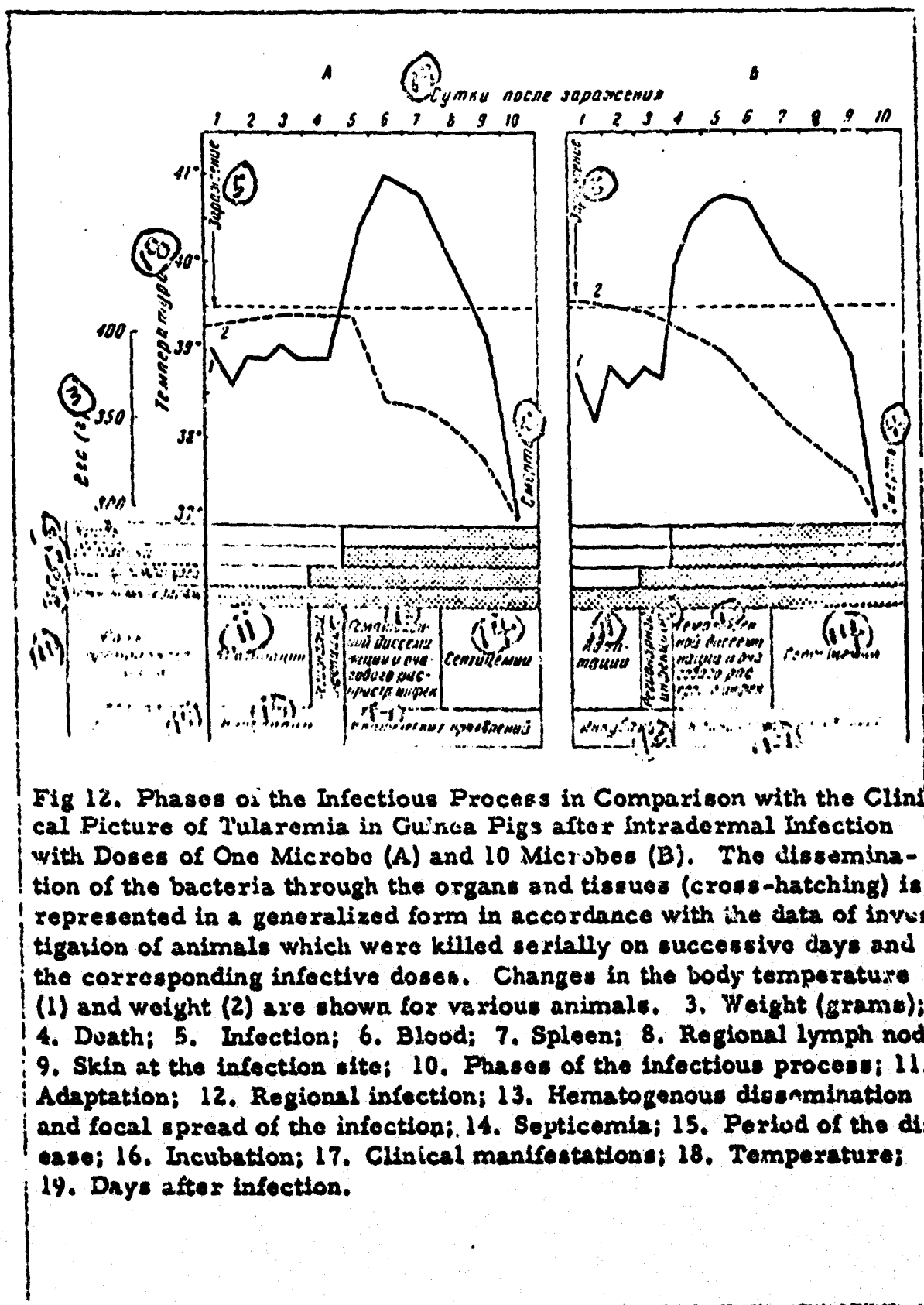


Fig 12. Phases of the Infectious Process in Comparison with the Clinical Picture of Tularemia in Guinea Pigs after Intradermal Infection with Doses of One Microbe (A) and 10 Microbes (B). The dissemination of the bacteria through the organs and tissues (cross-hatching) is represented in a generalized form in accordance with the data of investigation of animals which were killed serially on successive days and the corresponding infective doses. Changes in the body temperature (1) and weight (2) are shown for various animals. 3. Weight (grams); 4. Death; 5. Infection; 6. Blood; 7. Spleen; 8. Regional lymph node; 9. Skin at the infection site; 10. Phases of the infectious process; 11. Adaptation; 12. Regional infection; 13. Hematogenous dissemination and focal spread of the infection; 14. Septicemia; 15. Period of the disease; 16. Incubation; 17. Clinical manifestations; 18. Temperature; 19. Days after infection.



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growth of the culture occurs only when no less than 10,000 microbes are present per gram of investigated tissue. Bacterioscopy of the smears with this number of bacteria in the tissues is always negative. Approximately the same results were obtained from the study of organs of white mice and white rats (see Table 15).

During the phase of septicemia seeding of the splenic tissue exceeds that of the regional lymph node (see below). Apparently, the latter should be connected with the active necrotization of the lymph node tissue which limits the further multiplication of bacteria. In the regional lymph node (or nodes), aside from a marked enlargement of it, the most far-advanced changes of the tissue in the form of extensive caseous necrosis are always found (at the time of death of the animal), while the distant lymph nodes are moderately enlarged and only tissue hyperplasia and solitary granulomas are noted in them. These differences between the lymph nodes affected primarily or secondarily are so characteristic of guinea pigs that even at autopsy the routes of spread of the infection can be determined almost without error (after small doses of the infection). In the lymph nodes distant from the primary focus (not connected with one another by a common lymph flow) the tularemia bacteria penetrate even in the phase of hematogenous dissemination or septicemia. During this period of the disease the bacteria can penetrate into the lungs hematogenously or lymphogenously and can cause foci of secondary pneumonia, which in guinea pigs is observed in approximately 50 percent of the cases (Z. D. Khakhina).

Characteristic of the phase of septicemia is the regular presence of bacteria in the blood and progressive multiplication of them in organs rich in reticuloendothelial cells. Tularemia bacteria multiply mainly in the spleen, lymph nodes, liver, bone marrow and lungs. In this phase the number of bacteria in the spleen and other foci of active multiplication exceeds 1,000,000 per gram of tissue and is regularly found by culture (see Table 15) but not by bacterioscopy. In the internal organs, from the time of penetration of the tularemia bacteria into them, specific foci of inflammation in the form of granulomas eventuating in necrosis occur along with the degenerative changes in the parenchymal cells -- fatty degeneration and areas of necrosis.

In the case of a multiple granulomatous process necrosis can include considerable areas of tissue, and macroscopically these areas look like grayish nodules or small foci of irregular shape in the organs. The granulomatous process develops most actively in organs in which maximum bacterial multiplication occurs: in the spleen, lymph nodes, liver, etc. In the phase of septicemia necrotic changes increase; the defense forces of the body decrease; injury to the organs

and associated functional disorders progress rapidly, which leads to the death of the animals. We agree with the opinion of V. S. Kolesnik (1946) that in tularemia septicemia in guinea pigs "designates complete exhaustion of the phagocytic and bactericidal power of all the reticulo-endothelial elements associated with the blood stream and is expressed in the formation of multiple foci with active multiplication of microbes in the internal organs".

In guinea pigs infected intradermally with a dose of 10 microbes the number of bacteria at the time of death reaches 10,000,000 per gram of tissue of the regional lymph node; 100,000,000-1,000,000,000, of the spleen; and in one cc of blood, 100-10,000. The sequence of spread of tularemia bacteria and the increase in their numbers by organs and tissues of the guinea pig during the course of development of the infectious process are shown schematically in Figs 13 and 14.

It is perfectly obvious that with increase in the dose or change in the method of administering the bacteria the time of development of the various phases of the infectious process in guinea pigs will be shortened (like the entire process as a whole), and even the initial phases can entirely drop out, for example, after intravenous infection. In the case of subcutaneous injection of a dose of 10,000,000,000 microbes of the virulent strain 503 into guinea pigs the infectious process with a fatal outcome was completed in a period of less than two days.

In the work of I. S. Tinker and M. S. Drozhevskina (1948) different times for the development of various phases of the infectious process in guinea pigs after subcutaneous infection with a virulent strain of tularemia bacteria are mentioned from the ones we obtained. Unfortunately, the authors do not report the doses they injected into the guinea pigs but evidently they were quite massive. If three hours after beginning the experiment the authors found 6,000 bacteria at the injection site in the animals and after five-six days the guinea pigs were in an agonal state. For the purpose of finding the bacteria in the tissues the authors used cultures on liquid egg yolk medium, which is inferior in its sensitivity to the biological method (which we used in the experiments presented). This must be the explanation for the fact that after comparatively massive infection of the guinea pigs the authors were able to note a generalization of the infection only three days after the infection.

After infection of guinea pigs with museum strains (which have undergone attenuation to varying degrees) the infectious process can acquire a chronic nature (death on the 15th-20th day or later). Thereby, the seeding of organs and tissues with tularemia bacteria is much less than in animals infected with fully virulent strains, and in the tissues with progressive degenerative changes, beginning with the

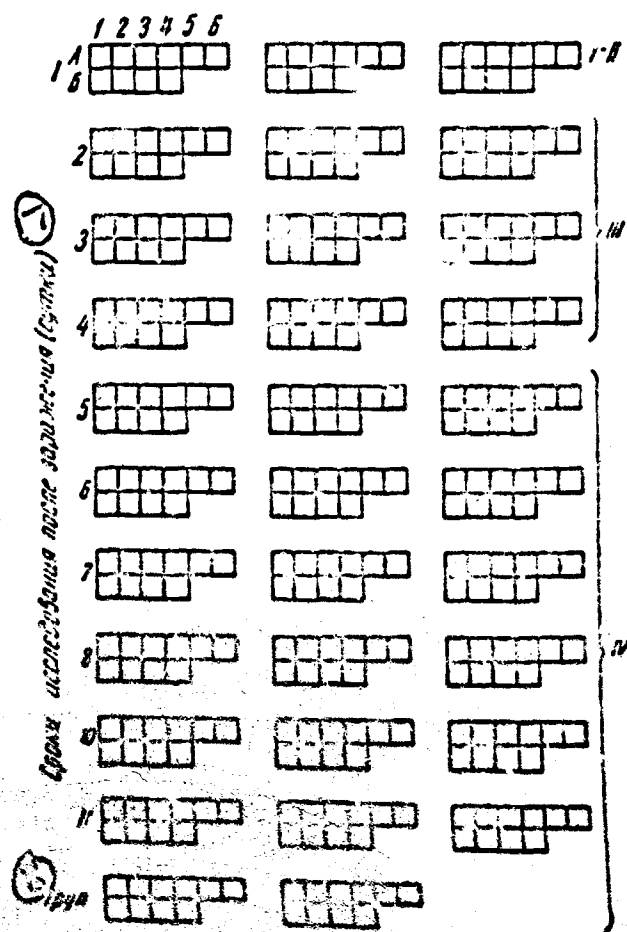


Fig 13. Results of Biological and Bacteriological Study of Guinea Pig Organs and Tissues at Various Periods after Subcutaneous Infection with a Dose of 10 Microbes of a Virulent Tularemia Strain, 9. Each figure designates an animal examined. A. Biological tests (on white mice); B. Cultures. 1. Lymph nodes; 2. Spleen; 3. Liver; 4. Blood; 5. Kidneys; 6. Bone marrow. Positive results are hatched. The Roman numerals on the right designate phases of the infectious process (see Fig 23). 7. Time of investigation after infection (days); 8. Carcass.

10th-12th day of the disease compensatory changes of a productive nature are found (A. V. Mashkov, 1953).

Bacterioscopy of the sinears from organs of guinea pigs dying of tularemia is usually positive only in those cases where the

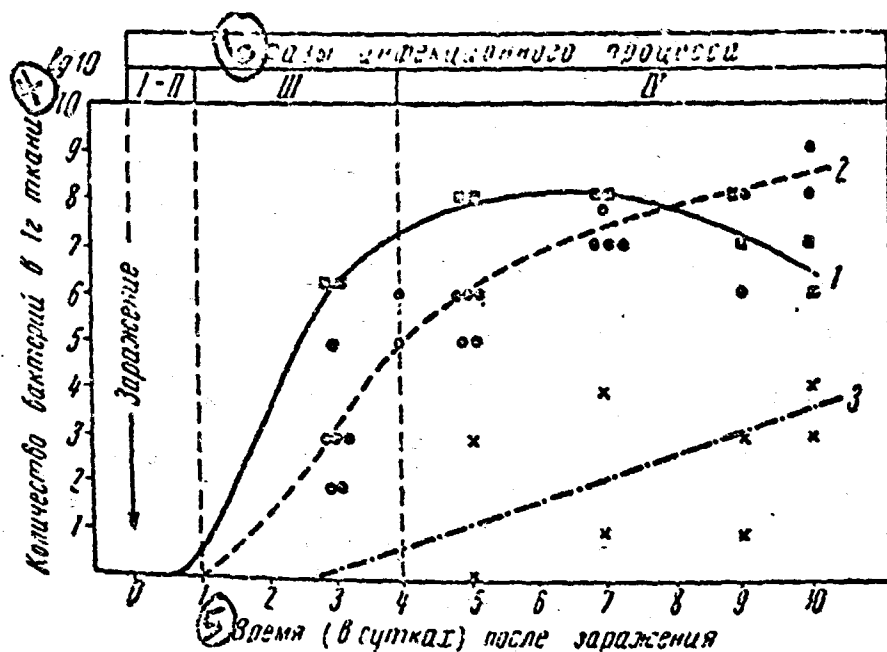


Fig 14. Change in the Number of Tularemia Bacteria per Gram of Tissue of the Regional Lymph Node (1, rectangles), Spleen (2, circles) or per cc of Blood (3, crosses) in Guinea Pigs infected Subcutaneously with a Dose of 10 Microbes of a Virulent Tularemia Strain, 9. The number of bacteria was determined by the method of titration on white mice. The figures shown are the actual data; the lines are extrapolations. 4. No. of bacteria per gram of tissue; 5. Time (in days) after infection; 6. Phases of the infectious process.

animals die in a short time after infection (before the sixth day), which is noted after the injection of large doses of a virulent culture. Bacteria are found in the lymph nodes, spleen and, less often, in the liver, and in various cases the number of them is estimated at +++ or ++++ (for the scale see Chapter VIII). Rare cases are known where in guinea pigs dying in a short time after massive doses of a virulent strain (the injection of a suspension from spontaneously infected mature ticks) tularemia bacteria were also found by bacterioscopy with a +++ grading in the blood (N. G. Olsuf'yev and Ye. N. Tolstukhina, 1949). Special studies showed that microscopic detection of tularemia bacteria in these smears from guinea pig organs is reliable only when the organs are seeded with more than 1,000,000,000 microbes per gram of tissue, which is associated with a very small size of the bacteria (T. N. Dunayeva). In guinea pigs which died after relatively long periods (on the ninth day or later), which was usually associated with minimum doses,

bacterioscopy of the smears from the organs is negative. The possibility of detecting tularemia bacteria in guinea pig organs by the method of bacterioscopy has been pointed out by many authors (G. Ya. Sinay, L. M. Khatenever and L. A. Levchenko, 1936; O. S. Yemel'yanova, 1951; Lilly and Francis, 1937, etc.).

Cultures on egg yolk medium are positive (if no contamination with extraneous flora has occurred), regardless of the dose of infection and the time of death, from the spleen, liver and regional lymph nodes, whereas from the blood growth is constant only in animals which die in a short time after the infection. The time of occurrence of growth in the cultures from organs and tissues of guinea pigs dying of tularemia varies in accordance with the degree of seeding of them with bacteria; in various cases growth appears in blood cultures for 10-12 days. These data are evidence to the effect that in tularemia in guinea pigs the bacteriological method of investigation of organs is much more "sensitive" and reliable than the bacterioscopic method, but both of them are much inferior to the biological method of examination. For obtaining a culture from a tularemia-infected guinea pig it may be killed in an agonal state, without awaiting the time of death, because cultures from the carcasses which have lain around can be contaminated by extraneous bacteria. Considering the possibility of various failures with the cultures part of the guinea pig organs should be preserved (in the cold) for the purpose of infecting a white mouse if necessary, in which, because of the much higher seeding of the organs, the finding of tularemia bacteria is readily possible both by culture and bacterioscopy.

The precipitation test with a heat extract from organs of animals dying of tularemia is usually negative because of the inadequate concentration of the antigen extracted.

The marked vascular permeability changes (exudation) observed in guinea pigs during the course of development of the infection, the degenerative processes in the parenchymatous cells, as well as the development of granulomas eventuating in necrosis clearly show that tularemia bacteria possess an irritant effect and produce toxins which are responsible for deep-seated tissue injuries. Rapid necrotization of the granulomas showed that in guinea pigs tissues are very sensitive to tularemia toxins. A. F. Mashkov (1952) injected guinea pigs with massive doses of formalin-killed tularemia bacteria and at the site of injection and in the internal organs he found specific granulomas as well as phenoma of necrobiosis which were considered by the author the result of the action of toxins of the bacterial cells. Data on the toxin of the tularemia microbe are presented in Chapter III.

A. G. Kratinov and coauthors (1946) determined the fact

that in guinea pigs at the time of death from tularemia a considerable ascorbic acid deficiency is found which is particularly marked in the suprarenal glands, spleen, lungs and regional lymph nodes; also, a reduction in the capillary resistance is noted. The authors conclude that in tularemia a toxic vitamin C deficiency occurs in the guinea pig organism. They determined the fact that keeping guinea pigs on a scorbutogenic diet considerable reduces their resistance to infection caused by attenuated tularemia strains. These data should be taken into consideration in experimental work on guinea pigs, particularly in immunological experiments, and one should watch the completeness of nutrition of the animals carefully, particularly in the wintertime, because otherwise the results of various experiments may be different.

**Characteristics of Tularemia with Other Methods of Infection.** Guinea pigs show almost the same high degree of susceptibility to aspiration infection as to subcutaneous and intradermal infection; somewhat less, to conjunctival, and least to alimentary and percutaneous (without scarification) infection (Fig 15).



Fig 15. Infectibility of Guinea Pigs with Tularemia by Different Methods of Administration of the Pathogen: Subcutaneous (1), Intradermal (2), Aspiration (3), Conjunctival (4), Alimentary (5), Percutaneous without Scarification (6); 7. Dose of microbes; 8. Thousand(s); 9. Million(s); 10. Billion.

In the experiments of R. A. Savel'yeva and G. P. Uglovoy

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it was determined that aspiration of single tularemia bacteria leads to infection and death of part of the guinea pigs used in the experiment, whereas the aspiration of scores of bacteria leads to the death of all the experimental guinea pigs after 10-13 days. In the former case infection was achieved as the result of an hour's stay in a chamber in which every 10 liters of air contained one microbe (according to the GKI standard), on the average; and in the latter case, 10 microbes. The probable number of bacteria aspirated by the guinea pigs was determined from the respiratory minute volume of the lungs of these animals (with consideration of the weight of the latter) and the time of exposure in the chamber. These authors determined the fact that after spraying a finely dispersed bacterial suspension into the air the bacteria penetrate into the most distant (with respect to the bronchi) parts of the guinea pig lungs; immediately after aspiration infection with a dose approximately equal to 5,000 microbes bacteria were found in the tissues of the guinea pigs excised from the surfaces of peripheral parts of the lung. In this case the infectious process can begin immediately in the lung tissue, developing in a manner similar to primary pneumonia. After 24 hours bacteria were found in the lungs and in the tracheobronchial lymph nodes, whereby the number of bacteria per gram of lung increased by approximately 10 times, reaching 1,000. After two days, bacteria in various cases was found in other organs also, for example, in the spleen. Beginning with the fourth day and later and until the time of death bacteria were found in all organs and tissues including the blood, whereby the number of bacteria increased sharply: for example, in one gram of lung there were 1,000,000-10,000,000 bacteria on the fourth day, and at the time of death of the animal (after eight days), 1,000,000,000-10,000,000,000.

In guinea pigs infected by aspiration, fine moist rales indicating pneumonia (R. A. Savel'yeva) are heard well in the lungs at the climax of the disease. The lymph nodes on the outside of the body remain unchanged. At autopsy in the guinea pigs which died a marked enlargement of the lungs, hyperemia and hepatization of the tissue were found, including considerable parts of the lung, necrotic nodules, which sometimes became confluent into extensive necrotic areas, marked enlargement (to the size of a pea or bean), densification and necrotization of tracheobronchial lymph nodes as well as changes in other organs typical of tularemia -- spleen, liver, etc. The cervical and submaxillary lymph nodes were not notably enlarged but were congested.

Primary tularemic pneumonia can be reproduced in guinea pigs by means of endotracheal administration of a bacterial suspension also (T. G. Linnik, 1946; V. S. Kolesnik, 1946). V. S. Kolesnik (1946) and Z. D. Khakhina (1948) point out that after endotracheal or

aspiration infection the primary inflammatory changes are found in the alveoli, alveolar septa and bronchioles, which can be considered a reaction to the site of incorporation and initial multiplication of the bacteria. These authors described in detail all the successive stages of development of tularemic pneumonia in guinea pigs.

In the case of conjunctival infection (by means of instillation of a bacterial suspension into the conjunctiva with the lid retracted without subsequent incision) in our experiments (N. G. Olsuf'yev and T. N. Dunayeva) the minimum full lethal dose (MLCD) amounted to 1,000 microbes for guinea pigs, that is, it was 1,000 times more than after subcutaneous infection. In the infected guinea pigs there was a typical ophthalmic form of tularemia characterized by a marked edema of the lids, hyperemia of the scleral blood vessels and lid mucosae, opacification of the cornea, purulent exudation from the eye and a marked enlargement of the posterior submaxillary and cervical lymph nodes on the side of the infection. The nodes could be found by means of palpation during the last few days of life of the guinea pigs. At autopsy of the guinea pigs, in addition to the changes indicated on the part of the eye and regional lymph nodes which at the time of death were sometimes enlarged to the size of a bean and were necrotic in depth, typical lesions of the internal organs were found.

The relative resistance of guinea pigs to conjunctival infection (without injury to the mucosa) attracts attention. Evidently, the mucosa of the eye is to some degree protected against the entrance of bacteria into it if their number is small. Relative protection of the eye against penetration of tularemia bacteria is also confirmed by experiment of M. P. Tereshchenko (1954) on white mice. These facts, to some degree, can explain the fact that the ophthalmic form of tularemia is not frequently encountered clinically. However, this problem needs further study.

Guinea pigs show considerable resistance to alimentary infection, studied in detail by R. A. Savel'yeva (1958) which is associated with the bactericidal properties of the gastric juice because of the presence of hydrochloric acid in it (R. A. Savel'yeva, 1956). On feeding guinea pigs infected food (pieces of white bread) or on instillation of a bacterial suspension (in physiological saline solution) into the mouth the MLCD proved to be equal to 100,000,000 microbes. Only part of the guinea pigs was infected from smaller doses, whereby a dose of 1,000 microbes did not produce infection. Alimentary administration of tularemia bacteria in some cases led to the occurrence of an anginal-bubonic form characterized by a marked enlargement, inflammation and partial necrosis of the submaxillary and cervical lymph nodes, usually unilateral, whereas in the gastric and intestinal



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mucosae there were no visible changes; the mesenteric lymph nodes were not enlarged. In other cases the abdominal form of tularemia developed with the presence of portals of entry of the infection in the mucous membrane of the small intestine and less often, of the stomach and a corresponding involvement (enlargement and partial necrosis) of mesenteric lymph nodes.

In part of the guinea pigs dying as the result of alimentary infection a combination of the anginal-bubonic and abdominal forms of tularemia was noted, which indicated simultaneous incorporation of the pathogen in the area of the oral or pharyngeal mucosa and the mucosa of the gastrointestinal tract. In one guinea pig an enlargement of tracheobronchial lymph nodes was observed with the absence of noticeable changes in the mesenteric or cervical and submaxillary lymph nodes. It is very possible that in these cases the bacteria penetrated into the tracheobronchial nodes from the esophagus, as Kh. Kh. Planel'yev and co-workers (1950) believe on the basis of their work on the Breslau strain of *S. paratyphi* in mice. However, after alimentary infection of guinea pigs with tularemia this route of penetration of bacteria is not characteristic. This problem needs further study.

In percutaneous infection (without scarification of the skin) of guinea pigs the MLCD proved to be equal to 10,000,000 microbes (N. G. Olsuf'yev and O. S. Yemel'yanova). In the majority of animals inflammatory changes (hyperemia and infiltration with subsequent formation of small pustules) typical of tularemia were found in the skin at the site of infection, and in the groin or axilla a bubo was formed, but in two cases (out of 20) no inflammatory skin changes were found either during life or at autopsy in the presence of the regional bubo. These cases can be considered "a purely bubonic" form of tularemia. After cutaneous application of even such a massive dose as 1,000,000,000 microbes death of the guinea pigs was observed after a relatively long period (seven-10 days), and this can serve as an indication of the fact that a small number of bacteria actually penetrated into the skin. The site of their penetration was apparently the hair follicles and sebaceous glands. At autopsy of guinea pigs typical lesions were found in the internal organs. These experiments showed that infection with tularemia bacteria can be accomplished through the normal intact skin, confirming the data of O'Hara (1925), Francis (1929), Downs and co-authors (1947) and other authors.

In the experiments on guinea pigs presented which used aspiration, alimentary and other methods of infection, in all cases the minimum infective dose (MID) was the same as the minimum lethal dose (MLD). We repeatedly checked the surviving animals for the presence of immunological reactions in them, investigated their organs

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by means of a biological test on mice, made cultures, etc., but in all cases a negative result was obtained attesting to the absence of infection in these animals.

The results of all the experiments can be summarized in the following way: 1. With different methods of administration of the virulent tularemia bacteria guinea pigs show different degrees of susceptibility to infection, greatest after subcutaneous, intradermal and aspiration methods, and somewhat less after conjunctival administration; least, by the alimentary and percutaneous (without scarification) methods. 2. After infection with fully virulent strains the guinea pigs are so sensitive to tularemia that the minimum infective dose for them coincides with the minimum lethal dose regardless of the method of injection of the bacteria (resp. "portal of infection"). Rare exceptions depend on the altered sensitivity of the guinea pig organism, associated with some previous antigenic effect (specific or nonspecific). 3. Each of the methods of infection has its own primary lesion localizations, usually determinable without difficulty by the degree of expression of the inflammatory process in the tissues at the site of incorporation of the pathogen and the degree of involvement of the regional lymph node. 4. In the case of methods of infection which are similar to the natural mode, in guinea pigs the following experimental forms of tularemia are clearly distinguished by the localization of the primary lesions: ulcerative-bubonic, purely bubonic, pneumonic, abdominal, anginal-bubonic, and ophthalmic-bubonic, which confirms the correctness of the previous clinical classification of these forms. 5. After any of the methods of infection tularemia proceeds in the guinea pigs as an acute septic disease causing untypical pathological and histopathological changes in the internal organs, such as the spleen, liver, suprarenal glands, lymph nodes, vascular system, and others.

Immunity. After infection of guinea pigs with fully virulent strains cases of recovery from tularemia are rare and are observed only after the use of minimum doses with the intradermal method of injection. We observed such cases and an analysis of them showed that guinea pigs with an altered (reduced) infectious sensitivity (see above) had been used for the infection. With other methods of infection we did not note any recovery of guinea pigs. With the usual acute course of the infection, ending in a fatal outcome, immunological reactions in guinea pigs such as the allergic reaction or the agglutination test are not found, which in our laboratory was checked specially on a large number of guinea pigs. However, guinea pigs quite often recover when they are infected with minimum doses of partially attenuated strains. In such a case, after recovery, which sometimes occurs after a long time, immunity is found in the guinea pigs which is

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adequate to withstand a subsequent infection with 100 or more lethal doses of a highly virulent strain. Subacute and chronic forms of the disease in guinea pigs can be diagnosed beginning with the seventh-10th day by means of the intradermal allergic test (V. A. Bychkov and L. G. Rappoport, 1931), as well as the agglutination test. A month after infection the serum titers can reach 1:320, less often, higher. In various cases allergic reorganization of the body and antibodies can be found in guinea pigs which have a subacute course of the disease and die after a long time. Evidently, in these cases the action of the immune forces of the body is inadequate to stop the further injury of tissues of the vital organs and the associated severe functional disorders of them.

In guinea pigs which have had tularemia the elimination of virulent bacteria from the body occurs slowly, and the animals can remain bacterial carriers for a long time: according to our observations, as long as 110 days (unlimited observation); according to the data of I. N. Mayskiy (1953), as long as eight months. Here, as in the subsequent presentation, by "bacterial carriage" we mean local carriage, without egress to the environment, the finding of a small limited number of tularemia bacteria in various tissues, usually in the lymph nodes, during the period of subsidence of the infection. Problems of immunity production and its characteristics in guinea pigs, learned chiefly from the effect of attenuated (vaccine) strains on their bodies, will be analyzed in Chapter IX.

#### Experimental Tularemia in White Mice

White mice possess a maximally high susceptibility and infectious sensitivity to tularemia, which makes them irreplaceable for use as biological test animals. When infected with fully virulent strains of tularemia bacteria the infectious process occurs in the mice acutely and leads to a fatal outcome in all cases without exception, even with the smallest doses, whereby the organs and tissues are massively seeded with the pathogen. However, just as in experiments on guinea pigs, the minimum infective dose by different methods of administration of the tularemia bacteria to the mice differs considerably, while the development of the infectious process has its own characteristic features.

#### Characteristics of Tularemia after Subcutaneous Infection.

The subcutaneous method of infecting white mice is used most often in laboratory practice; therefore, the infectious process produced by it has been best studied. In experiments on white mice the intradermal method of infection is practically equivalent to the subcutaneous method;

minor differences are noted only in the time of death of the animals, being later after the intradermal injection of bacteria (M. P. Tereshchenko). In the subsequent description we shall deal only with data obtained from subcutaneous infection. The minimum full lethal dose (MLCD) of a fully virulent strain amounts to one microbe according to the GKI standard. A dose of 0.1 microbe causes the death of a little more than 50 percent of the mice, that is, in practice it can be considered the LD<sub>50</sub> (Table 11).

Table 11

Mortality Rate of White Mice after Subcutaneous Injection of a Minimum Dose of 0.1 Microbe (Dilution 10<sup>-10</sup>) of a Virulent Strain

1 Автор	2 Штамм	3 Количество зараженных мышей	4 Количество мышей, погибших от туляремии	5 Процент погибших мышей
6 М. П. Терешченко (1954)	9	101	52	52
7 Т. Н. Дунаева . . . . .	9	112	63	56
8 Т. Н. Дунаева . . . . .	503	59	33	56
9 О. С. Емельянова . . . . .	503	222	127	57
10 Всего . . . . .		404	275	55

1. Author; 2. Strain; 3. No. of mice infected; 4. No. of mice which died of tularemia; 5. % of mice which died; 6. M. P. Tereshchenko (1954); 7. T. N. Dunayeva; 8. [Same as 7]; 9. O. S. Yemel'yanova; 10. Total.

This result, however, is obtained from impeccable performance of the experiments and keeping the experimental animals at the usual room temperature. It represents the average, but in various experiments deviations to one side or another are possible. Inadequate nutrition or keeping the mice at low temperature increases the mortality rate (M. P. Tereshchenko, 1956). A dose of 0.5 microbe after subcutaneous injection can cause the death of all experimental mice. However, from experiment to experiment the results can be irregular; therefore, this dose cannot be considered reliable as the MLCD. The minimum infective dose (MID) and the minimum lethal dose (MLD) of a virulent strain are the same for mice. Mice usually die after six-seven days from a dose of one microbe; after seven-eight

days, from a dose of 0.1 microbe, but in rare cases death can occur on the 12th-15th day and as an extremely rare exception, on the 18th day (T. N. Dunayeva). In the experiments of O. S. Yemel'yanova on 586 mice the largest number of animals died from a dose of 0.1 microbe after seven days (50 percent); from a dose of one microbe, after six and seven days (49 and 43 percent); from a dose of 10 microbes, after six days (79 percent). With increase in the infective dose the time of death of the mice comes sooner, which is seen from the experiment performed by Ye. M. Tsvetkova (Table 12).

Table 12

Relationship of Time of Death of White Mice to the Infective Dose (after Ye. M. Tsvetkova)

① Доза (микробыные и т.п.)	② сроки гибели зараженных мышей (сутки)				
0,1	—1	—1	7-0	7-0	7-0
1	7-0	7-0	6-0	6-0	6-0
10	6-0	6-0	6-0	6-0	6-0
100	6-0	5-0	5-0	5-0	5-0
1000	5-0	5-0	5-0	5-0	5-0
10 000	5-0	5-0	4-0	4-0	4-0
100 млн. ③	3-н	3-н	3-н	3-н	3-н

④: Мышь осталась жива.

1. Dose (microbes); 2. Time of death of infected mice (days); 3. Millions; 4. Mouse remained alive.

The initial stages of attenuation of the strains have little effect on their virulence for white mice; in any case, the dose of one microbe remains the full lethal dose, whereas in the case of guinea pigs and white rats the strain is notably less virulent. Only a delay in the time of death of the white mice to nine days or more (see Chapter III) is noted. Different colored laboratory strains of the house mouse (black, brown, etc.) as well as the wild strain are susceptible to tular-emia on a par with the white laboratory strain. According to the data of Bell and coauthors (1952), the age and sex of white mice exert no effect on the LD<sub>50</sub>.

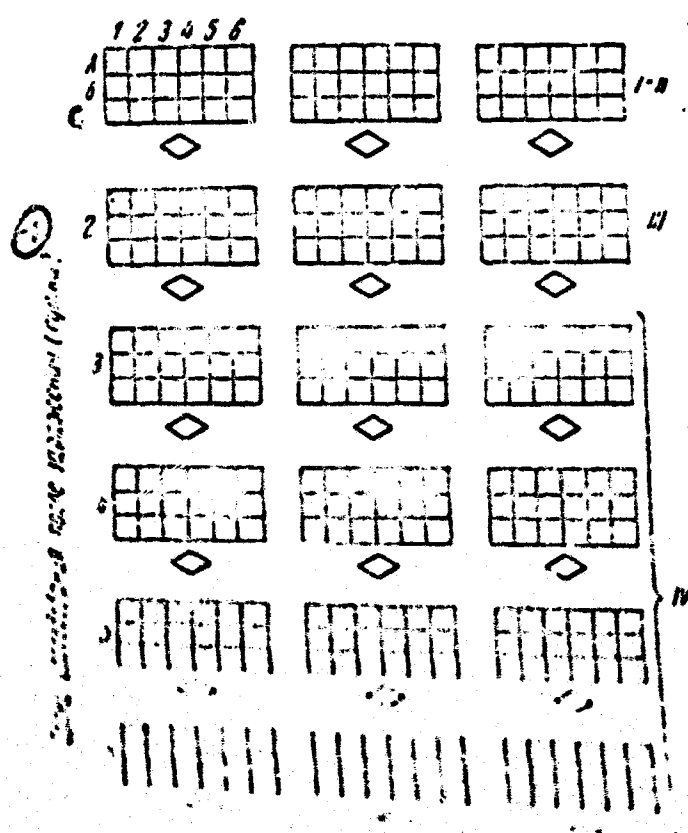
In mice, in view of their small size, clinical manifesta-tions of tularemia are difficult to detect. Usually, outwardly it is difficult to detect signs of the disease almost until the end of their

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lives. Only two-three days before death can it be noted that the hind paw corresponding to the side of infection is raised and a small infiltrate is found in the groin on palpation. At the end of life, the mice lose up to 15 percent of their weight. The agonal period, in contrast to guinea pigs, lasts a short time, sometimes a total of a few score minutes.

The development of the infectious process in the mouse organism undergoes the same phases as in the guinea pig organism, but the spread of bacteria occurs in a shorter time and is associated with a much more active multiplication. In the experiments of M. P. Tereshchenko and N. G. Olsuf'yev (1956), after subcutaneous infection of white mice with a dose of one microbe of a virulent strain, 9, the adaptation and regional infection phases lasted 24 hours; on the second day a transition of the infectious process to the phase of hematogenous dissemination and focal spread was noted; after three days, to the phase of septicemia, and the mice died after six days. Throughout the experiment it was possible to find bacteria regularly in the experimental mice by the biological method and with less regularity (beginning with the second-third day) by culture; bacterioscopy and the precipitation test were positive on examination of the mice only shortly before they died (Fig 16). It was clarified by T. N. Dunayeva that cultures from a piece of spleen from a tularemia-infected white mouse become positive only after the bacterial concentration per gram of tissue reaches 10,000-100,000 and are very rarely successful with concentrations of 100-1,000 microbes (Table 15).

With increase in the infective dose the development of the infectious process in the mouse organism is accelerated and correspondingly there is a shortening of the time in which the application of various methods of finding tularemia bacteria is effective. However, final seeding of the organs and blood by bacteria in mice, in contrast to guinea pigs, is more or less the same whether they die from small or large doses of infection. The rate of multiplication of tularemia bacteria in the bodies of white mice is great, particularly in phases of hematogenous dissemination and septicemia, at which time the number of tularemia bacteria in the infected tissues doubles approximately every two-and-a-half-three-and-a-half hours, and in 24 hours it increases by 100-1,000 times on the average (M. P. Tereshchenko and N. G. Olsuf'yev, 1956; T. N. Dunayeva) (Fig 17). The number of bacteria per gram of spleen in mice dying of tularemia can reach 100,000,000,000 (A. V. Mashkov and A. F. Taranenko, 1950; T. N. Dunayeva). With such a tremendous seeding of the spleen and other organs of the mouse with tularemia bacteria the latter are present in smears as almost a solid layer between the tissue elements and are



**Fig 16. Results of Examination of Organs and Tissues of White Mice at Different Periods after Subcutaneous Infection with a Dose of 10 Microbes with a Virulent Strain, 9. Each rectangle represents an examined animal (after M. P. Tereshchenko and N. G. Glsuf'yev, 1956).**  
**A. Biological tests (on white mice); B. Cultures; C. Bacterioscopy.**  
**1. Lymph node; 2. Spleen; 3. Liver; 4. Blood; 5. Kidneys; 6. Bone marrow; 7. The time of examination after investigation (days). The diamond designates the precipitation test (with tissue of spleen and liver); the hatched squares indicate the finding of tularemia bacteria. Roman numerals on the right are the phases of the infectious process.**

readily detected by means of bacterioscopy. The latter method gives fully reliable results in the diagnosis of tularemia in the mice but should, of necessity, be combined with culture. Unusually active multiplication of tularemia bacteria indicates complete defenselessness of

the organism of the white mouse to the infection caused by virulent tularemia bacteria, even if the infection was carried out with single microbes.

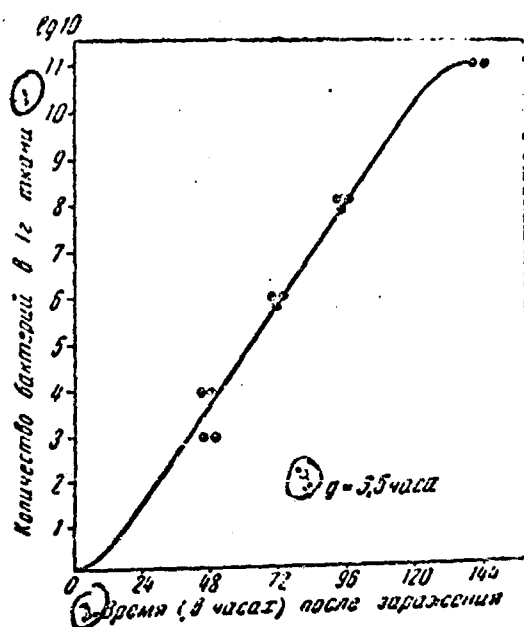


Fig 17. Changes in the Number of Tularemia Bacteria Per Gram of Splenic Tissue of the White Mouse Infected Subcutaneously with a Dose of 10 Microbes of a Virulent Tularemia Strain, 9. The number of bacteria was determined by the titration method on white mice. The rate of generation ( $g$ ) was calculated for the logarithmic phase of multiplication. 1. No. of bacteria per gram of tissue; 2. Time (in hours) after infection; 3.  $g = 3.5$  hours.

A. V. Mashkov and A. F. Taranenko (1950) point out that in white mice infected with virulent tularemia bacteria only exudative-degenerative changes are found histologically throughout the entire infectious process at the infection site and in the internal organs with no signs of the productive phase of inflammation. Morphologically the process is expressed in the formation of multiple granulomas which rapidly undergo necrotization in various organs. Bacterial multiplication occurs in organs rich in reticuloendothelial cells -- spleen, liver, bone marrow, lymph nodes and others, but not in the blood, the seeding of which occurs from the entrance of bacteria from these organs (I. S. Tinker and M. S. Drozhevskina, 1948). Believing that the pathological changes in the mouse organs are produced by toxic breakdown products of the pathogen cells in tularemia, A. V. Mashkov and A. F.



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Taranenko made appropriate investigations and determined the fact that inflammatory reactions in the form of specific granulomas develop in response to the injection of massive doses of killed tularemia bacteria in the bodies of mice, just as in guinea pigs.

It is difficult to assume that during the course of development of the infectious process caused by a virulent strain the mice manage to develop any immunological reactions even to a minimum degree. A. V. Mashkov (1952) did not find phagocytosis in mice throughout the infectious process.

In mice dying of tularemia quite characteristic pathological changes are found in the internal organs making it possible to suspect tularemia at autopsy in the majority of cases. At the injection site of the bacterial suspension under the skin there is usually quite a dense infiltrate. In the case of an inguinal localization the infiltrate is usually adherent to the adjacent inguinal lymph node. In such a case the latter is moderately enlarged but notably thickened, whereas the opposite inguinal lymph node is frequently enlarged less markedly and is notably congested. Usually, the axillary and retroperitoneal lymph nodes are enlarged, particularly on the side of infection. Hyperemia of the blood vessels of the subcutaneous tissue is marked, including the smallest ones. The spleen is enlarged and thickened, frequently of a pink-raspberry color; it is dryish on section (gives no scraping) and without visible necrotic nodules. The liver is enlarged and thickened with a clay-colored hue. The suprarenal glands are slightly enlarged and are frequently congested. The kidneys are pale. The small intestine in many cases is markedly congested over a considerable extent; the mesenteric blood vessels are congested. The lungs and heart show no notable external changes; small foci of hyperemia are found less often in the lungs. After infection with museum, partially attenuated strains hyperemia of the subcutaneous blood vessels in the mice which die is less pronounced; the spleen is markedly enlarged, dense, its color is cherry-red; the small intestine is not congested.

Everything presented on the pathogenesis of tularemia in white mice shows that cases of survival of mice after subcutaneous infection with an infective dose of a fully virulent strain are impossible. A latent infection accompanied by regional localization of bacteria is not characteristic of mice. Hence, the "multipassage" method with the use of white mice as biological tests in experimental work with virulent strains of tularemia bacteria or in isolation of such strains from nature is theoretically unsound. Cases described in the literature, where on biological examination of some object the infection in the mice used for the biological test began to be expressed only after several passages and the culture isolated from them was fully virulent,

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whereas the animals used in the initial passages remained outwardly healthy and cultures from them were negative, must be doubted. Factual verification of this matter showed the possibility of using the "multi-passage" method only in working with museum attenuated cultures, but not with virulent strains encountered under natural conditions (N. G. Olsuf'yev and M. P. Tereshchenko, 1957).

Characteristics of Tularemia after Other Methods of Infection. White mice are just as susceptible to aspiration infection as to subcutaneous infection; somewhat less, to intranasal and moderately or slightly susceptible to alimentary, conjunctival or percutaneous (without scarification) infection (Fig 18). In the experiments of R. A. Savel'yeva and G. P. Uglovoy on aspiration infection after an hour's exposure of the mice in a chamber, in which as the result of spraying a finely dispersed virulent strain, 503, approximately one microbe was present in every liter of air three out of five animals were infected and died of tularemia in a period of nine-12 days. According to calculations the infection occurred as the result of entrance of only single bacteria into the mouse lungs. Five guinea pigs which were in the chamber simultaneously with the mice were infected and died of tularemia in a period of 10-13 days. Such a result is explained by the fact that guinea pigs, by virtue of their larger size, could aspirate almost 20 times more bacteria into their lungs than the mice. In another experiment in which 10 bacteria were present in every liter of air and an hour's exposure was used all five mice were infected and died of tularemia. In all the animals which died, along with changes typical of tularemia in the spleen, liver and other organs, foci of hyperemia or widespread marked congestion of various lobes were noted in the lungs.

The susceptibility and infectious sensitivity of white mice to other methods of infection have been studied in detail by M. P. Tereshchenko (1954). In the comparative experiments of this author, conducted with the use of a virulent strain, No 9, it was determined that the MLCD for white mice after the intranasal method of infection amounts to 100 microbes; after the conjunctival and percutaneous methods (without scarification), 10,000,000; after the alimentary method, 1,000,000 microbes. After the application of a bacterial suspension to the scarified skin the MLCD was reduced by approximately 100 times (to 100,000 microbes). Data on the much lesser effectiveness of percutaneous (or the so-called "Austrian") method of infection by comparison with the subcutaneous are very important for laboratory practice, because they assist in avoiding errors in the performance of biological tests on mice.

Pathological changes in the mice which died after all methods of infection characterized the acute form of tularemia. After the

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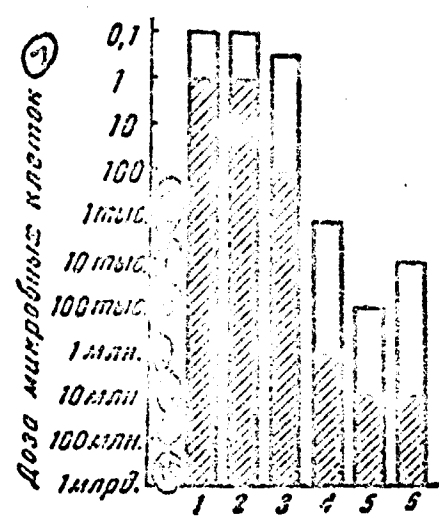


Fig 18. Infectibility of White Mice with Tularemia after Different Methods of Administering the Pathogen: Subcutaneous (1), Aspiration (2), Intranasal (3), Alimentary (4), Conjunctival (5), Percutaneous without Scarification (6) (after M. P. Tereshchenko, 1954, with additions). The key is the same as for Fig. 15. 7. Dose of microbes; 8. Thousand(s); 9. Million(s); 10. Billion.

intranasal method of infection inflammatory changes were found in the lungs, in addition to the other changes, and after the alimentary method there was intestinal and gastric hyperemia which was somewhat greater than with the other methods of infection. In all methods of infection in the mice which died a tremendous number of tularemia bacteria was noted in the spleen, liver, lymph nodes and blood. The intensity of septicemia did not depend on the infective dose. Cultures from the spleen and other organs on coagulated egg yolk medium succeeded without any difficulties if a fresh carcass was dissected.

In the experimental animals which survived M. P. Tereshchenko did not once find bacterial carriage or immunity to tularemia, that is, they were not infected. This gives us reason to believe that in all the methods of infection with a fully virulent strain of tularemia bacteria the minimum infective dose is simultaneously the minimum lethal dose for white mice. In view of the fact that survival (recovery) of the mice and the corresponding creation of immunity in them are possible only when they are infected with strains of tularemia bacteria which have been subjected to considerable attenuation this problem.

will be analyzed in Chapter IX.

### Experimental Tularemia in White Rats

In view of the low degree of infectious sensitivity to tularemia in them white rats are not suitable as biological test animals for detecting the pathogen of this infection by comparison with white mice and guinea pigs, and apparently, as the result of this for a long time they did not attract the attention of investigators. We have been using these animals since 1940 for determining the virulence of tularemia strains isolated from nature in parallel with tests on white mice (see Chapter III). In recent years, white rats have begun to be used for comparative pathological immunological studies also (A. V. Mashkov, T. N. Dunayeva, Downs, Larson, Foshay). The white rat organism is much more like the human organism in its relation to tularemia infection than the guinea pig or white mouse organism is. This justifies the utilization of white rats in experiments on comparative pathology and immunology of tularemia. We shall deal with the data obtained both on white and brown rats, since these animals represent the same species (*Rattus norvegicus* Berkenh.).

#### Characteristics of Tularemia after Subcutaneous Infection

After subcutaneous injection of a virulent strain the rats can be infected with the smallest doses of bacteria, but thereby a completely benign process develops in the animals which ends in recovery. A dose of one microbe (by the GKI standard) of a fully virulent strain is adequate to bring about infection of all rats used in the experiment, both white (O. S. Yemel'yanova, T. N. Dunayeva) and brown (A. A. Aysel, 1951), whereas a dose of 0.1 microbe is responsible for the infection of half of the animals used in the experiment (T. N. Dunayeva).

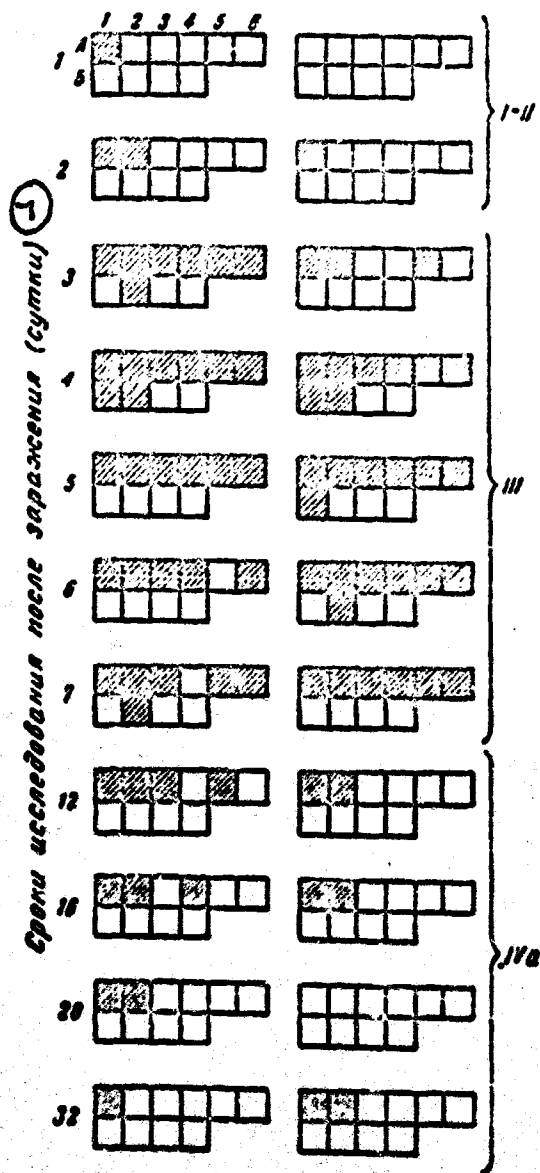
After subcutaneous injection of a dose of 10 microbes of a fully virulent strain (503) into white rats the development of the infectious process in the animals can be represented, according to the data of T. N. Dunayeva's experiments, in the following form (Fig 19). The adaptation phase of the microbe along with the phase of regional infection lasts about two days. On the third day a phase of hematogenous dissemination occurs accompanied by the spread of bacteria throughout the body and the occurrence of foci of multiplication of them in various organs. It is curious that this phase in the white rats is characterized by approximately the same degree of seeding of internal organs (spleen) as the analogous phase in guinea pigs. After this infective dose the maximum infection of the organs and tissues of the rats is observed between the fourth and sixth day after infection. At this time, in the regional lymph node and spleen 100,000-1,000,000 tularemia bacteria

are found per gram of tissue; in the blood, 100 bacteria (Fig 20). On the seventh day after the injection the infection begins to subside, which is expressed in a reduction in the number of bacteria in the main foci involved and a marked lessening of the entrance of bacteria into the blood. This turnabout is associated with the fact that at this time the immune reactions of the body begin to develop, preventing a transition of the phase of hematogenous dissemination to the phase of septicemia. The development of immunity is indicated by the appearance of antibodies in the blood on the seventh day which rapidly increase on subsequent days. The phase of subsidence of the infection lasts approximately 15-20 days after injection of the bacteria and is associated with successive elimination of tularemia bacteria from the organs. After the 20th day bacteria are found in small quantities chiefly in the regional lymph nodes and partly in the spleen. Clinically, at this time the rats have recovered, which can be judged by their gain in weight.

The comparatively slight seeding of the organs and tissues of white rats is characteristic also of infection of the latter with higher but not lethal doses of the pathogen (A. V. Mashkov, 1952). In the experiments of American authors (Downs and coauthors, 1949), in white rats infected subcutaneously with a dose of 3,600,000 bacteria (actual), the intensity of seeding of the spleen per gram of tissue reached 1,000,000,000 and that of the blood, 10,000 microbes, which is possibly associated with the greater virulence of the American strains.

According to the data of A. V. Mashkov (1952), tularemia infection in white rats after injection of sublethal doses occurs in the form of a granulomatous process which is of a benign character. In the internal organs the granulomas were found two-five days after infection, but no necrotic changes were noted in them throughout the entire observation period. The resolution of the inflammatory process began after the seventh day, and at the end of the observation period only residual phenomena were encountered in the internal organs in the form of the initial stage of encapsulation of granulomas and the formation of fibrillar connective tissue with minor inflammatory phenomena. Only in the spleen, along with the scarring granulomas were fresh granulomas encountered from time to time. The absence of necrotic changes in the granulomas formed in the internal organs of white rats as the result of infection with sublethal doses of fully virulent bacteria (strain 503) has also been noted by A. P. Gindin in his study of material obtained from us.

Woodward and coauthors (1954) determined the fact that after infection of white rats with virulent tularemia bacteria a reduction occurs in the amino acid concentration in the blood with a complete



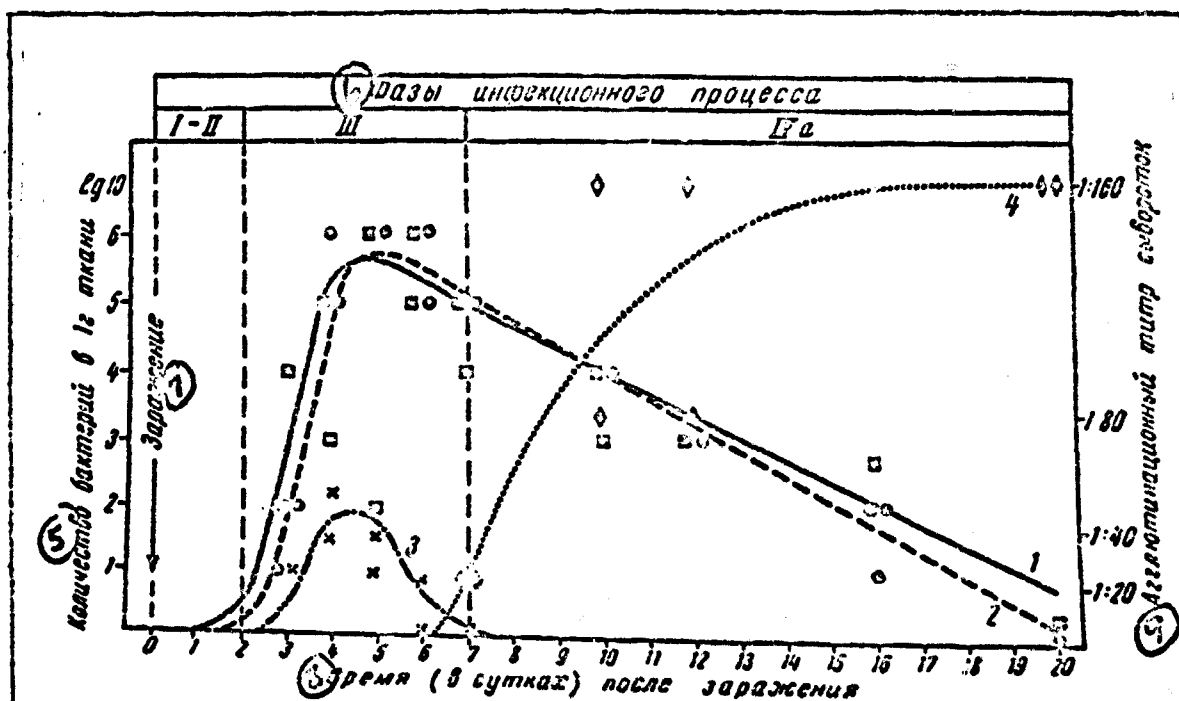


Fig 20. Change in the Number of Tularemia Bacteria per Gram of Tissue of a Regional Lymph Node (1, rectangles), Spleen (2, circles) and per cc of Blood (3, crosses) and the Accumulation of Agglutinins in the Blood (4, diamonds) in White Rats Infected Subcutaneously with a Dose of 10 Microbes of a Virulent Tularemia Strain, 9. The number of bacteria was determined by the method of titration on white mice. The figures represent the actual data; the lines, extrapolations. 5. No. of bacteria per gram of tissue; 6. Infection; 7. Phases of the infectious process; 8. Time (in days) after infection; 9. Serum agglutination titer.

confirmed by the fact that cystine, necessary for metabolism of this microbe, disappears from the animal's blood. The relation of the deviations in the quantity of amino acids in the blood of rats to the development of the infectious process is proved by the fact that in immune rats (those which have recovered) the injection of 100 LD<sub>50</sub> did not cause any changes in the amino acid content in the blood. The injection of killed virulent bacteria and living avirulent bacteria in a dose of  $3.5 \times 10^9$  microbes did not cause changes in the quantity of amino acids in the blood either.

After subcutaneous injection of bacteria the full lethal dose (MLCD) of a fully virulent strain usually amounts to 1,000,000,000



microbes (according to the GKI standard) for white rats weighing 100-150 grams. A dose of 100,000,000 microbes causes the death of only part of the rats, but it is greater than the LD<sub>50</sub>, whereas a dose of 10,000,000 microbes can be equal to the LD<sub>50</sub> or it can be less depending on the strain and other experimental conditions. Finally, the dose of 1,000,000 microbes usually causes the death of only a limited number of rats (Table 13). Doses of 100,000 microbes or less cause the death of only occasional rats.

Table 13

Mortality Rate of White Rats and Time of Death from Tularemia as a Function of the Infective Dose after Subcutaneous Injection of Virulent Strains of Tularemia Bacteria (after O.S. Yemel'yanova and T.N. Drayva)

① Доза заражения (микробы или мл)	Штамм 21			Штамм 9			Штамм 503		
	② число зараженных животных	③ число павших от туляремии	④ средний срок гибели (сутки)	② число зараженных животных	③ число павших от туляремии	④ средний срок гибели (сутки)	② число зараженных животных	③ число павших от туляремии	④ средний срок гибели (сутки)
1 млрд.	9	9	2,4	35	35	2,7	41	41	3,0
100 млн.	10	8	5,2	24	20	4,5	35	25	4,0
10 "	10	7	4,1	19	12	4,0	24	5	4,4
1 "	10	3	4,0	13	2	5,0	28	7	4,7
100 000	10	0	—	25	7	6,0	Опыты не ставились (10)		
10 000	10	1	5	25	2	8,0	То же (10)		
1 000	10	1	13	Опыты не ставились (10)			25	0	(10)
100	10	1	8	То же (10)			Опыты не ставились (10)		
10	10	1	20	25	3	7,0			
1	10	0	—	Опыты не ставились (10)					

Note. Strain 21 was tested in a single stage three months after isolation. Strains 9 and 503 were tested several times at various periods after isolation. In the Table the data of these tests, conducted with the aim of determining the virulence of the strains during the time they were kept in the laboratory, are summarized. For the purpose of preventing attenuation the strains were maintained by passages through white mice (strain 9) or guinea pigs (strain 503) and were kept in ampoules dried under vacuum conditions. 1. Infective dose (microb.); 2. Strain 21; 3. Strain 9; 4. Strain 503; 5. No. of animals infected; 6. No. which died of tularemia; 7. Average survival time (days); 8. Billion; 9. Millions; 10. Experiments not performed; 11. Same.



We have had repeated opportunities of isolating strains under natural conditions in which the MLCD for white rats amounted to 10,000,000 and in rare cases, 1,000,000 microbes.

At autopsy of the white rats which died of acute tularemia the marked congestion of blood vessels of the subcutaneous tissue, including the smallest, as well as the enlargement and densification of the liver and spleen with the absence of visible necrotic nodules in these organs attracted attention. The spleen is usually dryish on section. The lymphatic system shows no visible changes; only the lymph nodes regional to the site of infection can be somewhat enlarged or congested. The suprarenal glands in a number of cases can also be enlarged and congested. The small intestine frequently contains yellow or bloody contents which show through the walls. On death of the rats in the remote periods after infection the picture becomes non-characteristic; there is no marked congestion of the blood vessels of the subcutaneous tissue; the spleen and liver are flabby, slightly enlarged but without visible necrotic nodules; the lymph nodes are little changed, etc.

A relationship is noted between death of the rats and the infective dose, for example, after the administration of a dose of 1,000,000,000 microbes the average survival time of the rats for the three strains amounted to two-and-a-half-three days; from a dose of 1,000,000, four-five days. From smaller doses death of the rats can occur in rare cases before the 20th day, but sometimes cases in which the rats die after a long period (up to the 12th day) are noted also after the injection of such doses as 10,000,000 or 100,000,000 microbes.

Cultures from the spleen and other organs of rats which died of acute tularemia are usually positive, but sometimes, particularly on blood culture, growth in the form of isolated colonies appears on the medium on the fourth-fifth day or later, which indicates a small number of bacteria in the culture material. According to our data, bacterioscopy of organs of white rats which died of acute tularemia is negative in half of the cases (57 percent), while in positive cases bacteria are found in the organs and tissues (spleen, liver, lymph nodes, and rarely in the blood) usually only in moderate quantities (with a +++ evaluation according to the scale adopted). Tularemia bacteria are encountered in large numbers (++++) relatively rarely in rats which die and chiefly after the maximum infective dose (1,000,000,000). Biological examination of the organs of white rats dying of tularemia is inevitably positive, and the precipitation test is negative because of the inadequate quantity of antigen.

The injection of massive doses of the pathogen causes the development of an infectious process in the rats with the predominance

of signs of toxemia. This is indicated by rapid death of the animals as well as the characteristics of pathological changes of the organs and tissues and the sparsity of seeding of the latter with bacteria at the time of death. A. P. Gindin, who investigated the organs of white rats killed during the agonal period 50 hours after subcutaneous injection of a dose of 1,000,000,000 microbes of a fully virulent strain (503) of *B. tularensis*, at our request, found pronounced necrotic lesions of the tissues in the regional lymph nodes, spleen and liver of the animals with an almost complete absence of proliferative changes (granulomas). Tissue necrosis was noted also in the heart muscle and in the kidneys, and all this is evidence of the predominance of a picture of toxicosis and the suppression of the defensive reactions of the macroorganism. Toxicosis evidently occurs as the result of active destruction of a large number of tularemia bacteria injected into the rat under the influence of the natural defensive forces of the body, as well as by the accumulation of breakdown products of the latter. Thereby, not all the bacteria are destroyed, and part of them continues to multiply. If the rat organism succeeds in coping with the infection during the first four days after infection usually recovery occurs. Thus, in 68 cases where rats died of doses of 10,000,000 and 100,000,000 microbes of virulent strains 77 percent died in a period up to the fourth day inclusive. Cases of death in rats later than this time, apparently, are associated with the superimposition of additional infection or some other causes accounting for a weakening of the animal organism. However, when the animals survive later than the fourth day a typical septicemia develops in rare cases, and in the animals which die then bacterioscopy shows mass seeding with tularemia bacteria (++++) in all organs and tissues including the blood.

The initial stages of attenuation of tularemia bacteria have a rapid influence on their virulence for white rats, and such a dose as 1,000,000,000 microbes is no longer lethal after subcutaneous injection or else produces death of only a small part of the animals. Correspondingly, a dose of 100,000,000 microbes or less becomes non-lethal for rats (see Chapter III).

The data presented permit us to consider brown rats and the white laboratory strain of them highly susceptible but relatively insensitive to tularemia. The low degree of infectious sensitivity of white rats to tularemia is a species characteristic, even in young individuals. In the experiments of T. N. Dunayeva the majority of young rats weighing 40-90 grams withstood subcutaneous infection with a dose of 1,000,000 microbes (two out of 19 animals died of tularemia) on a par with adult rats, whereby no essential differences were noted in the intensity of seeding of the organs of the young and adult rats which died.

Characteristics of Tularemia after Other Methods of Infection. After intranasal injection of tularemia bacteria it is possible to infect brown rats in part of the cases with a fatal outcome after doses of 10,000-100,000,000 microbes (A. A. Ayseli, 1951). In the rats which died inflammatory changes were found in the lungs. Minimum infective doses remained unknown. In the experiments of T. N. Dunayeva, after infection of white rats by means of feeding a bacterial suspension in a piece of bread, it was determined that the minimum infective dose (MID) was equal to 1,000,000 microbes (two out of five rats were infected), whereas the full infective dose amounted to 100,000,000 microbes (all of five rats were infected). In all cases the rats survived and recovery of them from tularemia was determined bacteriologically (biologically) and by serological methods. The dose of 100,000 microbes did not cause infection of the rats. After alimentary infection of the rats a fatal outcome could be observed in them after the administration of a very massive dose, for example, after feeding the rats the white mice which died of tularemia (A. A. Ayseli, 1951). Nevertheless, various rats can survive even after eating three mice (when the mice weigh between 12 and 15 grams they can contain 10,000,000,000 microbes per gram of carcass, therefore, a total of 120-150,000,000,000 tularemia bacteria).

White rats show a relatively high degree of sensitivity to intraperitoneal infection: the LD<sub>50</sub> of a fully virulent strain amounts to 1,000 microbes for them; the MLCD, 100,000 microbes (T. N. Dunayeva).

Immunity. In rats which have recovered from tularemia a very strong immunity to reinfection is found as well as specific antibodies which can be demonstrated both in the agglutination test and in the complement-fixation test (A. A. Ayseli, 1951). In "normal", that is, nonimmune rats no antibodies are found against tularemia bacteria. Immunity which develops after recovery from tularemia is sufficient to prevent death in rats when they are given a subsequent injection of several full lethal doses of a virulent strain of tularemia bacteria. The formation of specific antibodies is observed in the rats when they are infected with massive as well as with small doses of the pathogen and serves as a reliable diagnostic sign of recovery of the animal from tularemia when examined at certain periods after infection. According to the data of T. N. Dunayeva, with small doses of a subcutaneous infection (one-1,000 microbes) agglutinins usually begin to be found in the blood of rats on the sixth-seventh day, but the serum titers at this time do not exceed 1:20. Antibody production reaches the maximum at the beginning of the third week after infection, then lessens, and after 40 days in part of the rats agglutinins are not found even in a serum

dilution of 1:5. In response to the injection of massive doses of bacteria (1,000,000-100,000,000 microbes), in cases of survival the rat organism responds with an accelerated and active antibody production, which reaches a maximum as early as the 10th day after infection; on the 15th day it begins to decrease, but on the 40th day agglutinins are found in all animals in serum dilutions of 1:5-1:80 (Fig 21). Evidently, differences are associated with the fact that in the latter case a large quantity of antigen enters the body at one time, and further accumulation of it from bacterial multiplication occurs in a short time, whereas in the former case antigen can accumulate during the course of development of the infectious process only later and, evidently, in smaller quantities than in the latter case. With a large infective dose the maximum antibody level is notably higher (average titer is 1:250) than after a small dose (1:130), which indicates the relationship of antibody production to the quantity of antigen. After intraperitoneal infection in rats high agglutination titers of the sera were observed (up to 1:160) even 50 days after infection with comparatively small doses of the pathogen (100-1,000 microbes). On the average, they amounted to 1:74. This indicates more complete survival of the bacteria in this method of infection, which is confirmed by the reduction in the MLCD.

In contrast to antibody production, a clear-cut allergic reaction of the skin to the injection of the tularemia antigen is not characteristic of white rats, and this method cannot be used for determining immunity to tularemia in rats (T. A. Kalitina, 1956; T. N. Dunayeva). Only in a few rats maximally immune to tularemia can a small infiltration of the skin be found without hyperemia 24 hours after the intradermal injection of tularin.

Elimination of tularemia bacteria from the bodies of rats which recover, as determined by the biological test method on white mice, occurs in a relatively short time, being completed by the 40th day in the great majority of rats (A. A. Ayseli, 1951; T. N. Dunayeva). However, in occasional rats this process of elimination of tularemia bacteria can drag on and may not be completed until the 117th day. The rarity of this phenomenon is proved by the fact that in 73 rats (57 brown and 16 white) a total of three cases of lingering bacterial carriage was recorded -- for more than 50 days (A. A. Ayseli). In the rats which recover from tularemia immunity to a second infection with a known lethal dose of a virulent strain continues to be maintained even after the organism is free of tularemia bacteria, for example, in the experiments of N. G. Olsuf'yev and coauthors (1957) immunity was studied up to six months (the observation period) in rats. Therefore, in white rats which have had tularemia immunity regularly goes into the post-infectious (sterile) phase, which is much longer lasting than its infectious phase.

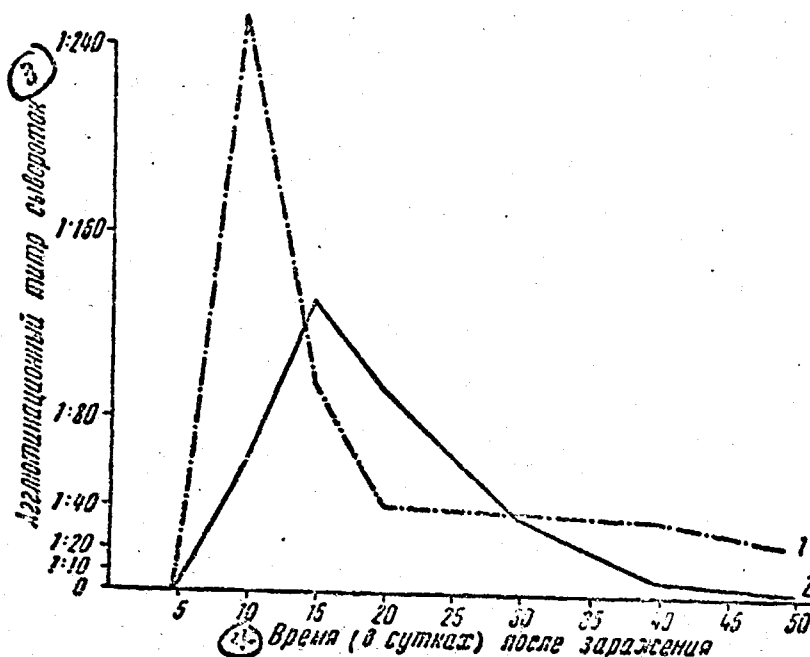


Fig 21. Dynamics of Agglutinins in the Blood of White Rats after Subcutaneous Infection with Doses of 1,000,000-100,000,000 (1) and 1-1,000 microbes (2) of a Virulent Tularemia Strain, 9; 3. Agglutination titer of sera; 4. Time (in days) after infection.

#### Experimental Tularemia in Rabbits

Even the first investigators of tularemia in the USSR, S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova (1928), directed attention to the lower sensitivity of rabbits to tularemia than guinea pigs and white mice. The possibility of reproduction of infection in rabbits accompanied by recovery even after relatively large doses of infection with a virulent culture as well as the convenience of observation of the development of the immunological reactions, particularly antibody production, attracted the attention of Soviet investigators to this species of animals as an experimental model for tularemia (A. A. Miller and N. K. Grzhebina, 1937; P. V. Somov, 1939; B. Ya. El'bert and N. A. Gayskiy, 1941; L. M. Khatenev, 1943; V. P. Dzhanpoladova, 1948 and others). A major obstacle in working with rabbits is their frequent infection with pasteurellosis, coecal and other infectious diseases, which, occurring in a latent manner, are exacerbated

under the influence of tularemia infection and distort the results of the experiment.

Characteristics of Tularemia in Subcutaneous and Intra-dermal Methods of Infection. After the infection of rabbits with moderate and small doses of a fully virulent strain of tularemia bacteria the animals usually recover. A dose of one microbe of a fully virulent strain after subcutaneous and intradermal injections is infective for all animals used in the experiment (T. N. Dunayeva). The possibility of subcutaneous infection of domestic rabbits with a dose of one microbe has been pointed out in the work of P. V. Somov (1939).

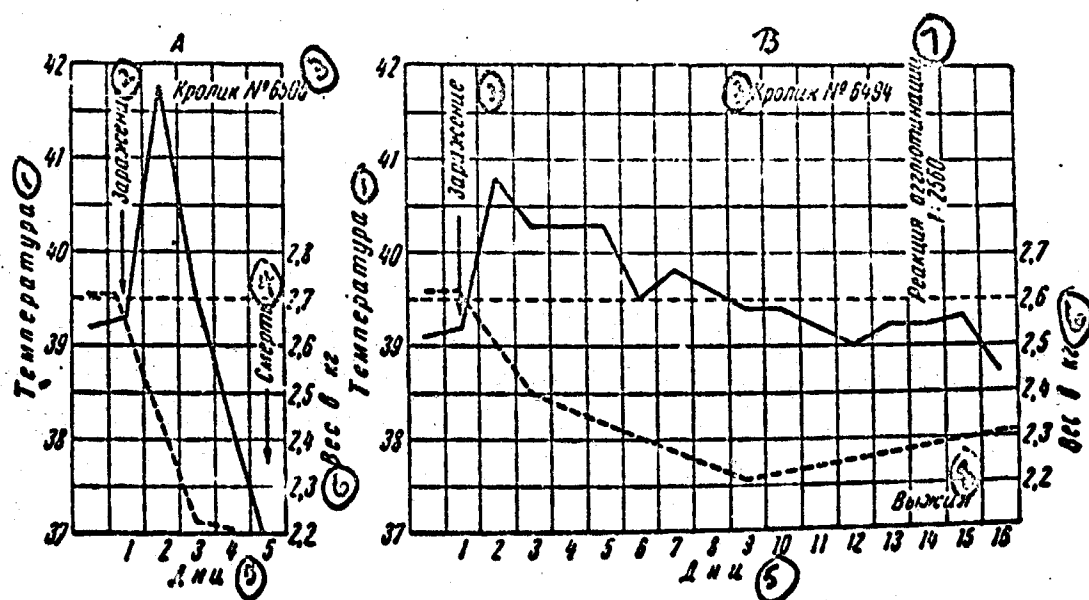


Fig 22. Temperature Changes (Solid Line) and Weight Changes (Broken Line) in Domestic Rabbits after Subcutaneous Infection with a Virulent Tularemia Strain. A. With an acute course of the disease (with a fatal outcome) after the administration of a dose of 1,000,000,000 microbes. B. With a subacute course of the disease (eventuating in recovery) after injection of a dose of 100,000,000 microbes. 1. Temperature; 2. Infection; 3. Rabbit No; 4. Death; 5. Days; 6. Weight in kilograms; 7. Agglutination test; 8. Survived.

In the infected rabbits an objective expression of the disease is an elevation of body temperature (found on rectal measurement) and a decrease in weight, and during the recovery period, the finding of specific antibodies which are maintained for a long time (Fig 22). According to the data of T. N. Dunayeva, after subcutaneous infection

with a dose of 10 microbes the elevation of body temperature (above  $39.5^{\circ}$ ) is noted in rabbits beginning with the third-fifth day after infection and is maintained three-six days, after which it drops to normal; the highest elevation of the body temperature is  $40.5^{\circ}$ . After infection with a dose of one microbe elevation of the body temperature is not observed in all rabbits and may be brief, sometimes only one day. After intradermal injection of minimum doses (10-100 microbes), along with elevation of body temperature at the application site, an infiltrate and hyperemia measuring 5x5 to 10x12 millimeters appear on the third-fourth day. The local reaction can be maintained for more than a month in the form of a slight densification with a small encapsulated necrotic focus. In rabbits infected with a dose of one microbe, inflammatory changes in the skin are less pronounced and are resorbed more rapidly, on the 10th-20th day. The bacteriology of the tularemic process in rabbits has not been studied after sublethal doses.

In the subcutaneous method of injection of bacteria a dose of 1,000,000,000 or 10,000,000,000 microbes of fully virulent strains is usually only the MLD but not the MLCD for rabbits weighing over two kilograms, that is, the animals sometimes survive after these doses (Table 14).

Table 14

Mortality Rate of Domestic Rabbits and Time of Death from Tularemia as a Function of the Infective Dose after Subcutaneous Injection of the Virulent 503 Strain of Tularemia Bacteria (after T.N. Danyeva and O.S. Yemel'yanova)

1. Доза заражения (микроорганизмы)	2. Число зараженных кроликов	3. Число погибших от туляремии	4. Срок гибели (сутки)	5. Средний срок гибели (сутки)
1 млн. 6	3	0	—	—
10 " 6	15	2	5, 7	—
100 " 6	8	2	3, 4	—
1 млрд. 7	8	7	3, 3, 3, 4, 5, 6, 9	4,7
10 " 7	13	12	2, 2, 2, 2, 2, 3, 4, 4, 4, 4, 7	3,3

1. Infective dose (microbes); 2. No. of rabbits infected; 3. No. which died of tularemia; 4. Survival time (days); 5. Average survival time (days); 6. Million; 7. Billion.



In the data presented the dose of 100,000,000 microbes amounted to the LD<sub>25</sub> but usually it is the LD<sub>50</sub>.

We know of only very few strains which caused the death of all animals used in the experiment in a dose of 100,000,00 (or higher) (see Chapter III, Table IX). Doses of 10,000,000 microbes or less cause the deaths of only occasional animals. After infection with large and small doses of the pathogen the possibility should be anticipated of occurrence of mixed infections (pasteurellosis and others) provoked by this infection. In such a case, a fatal outcome can occur after the injection of minimum doses of tularemia bacteria. After the subcutaneous injection of massive doses (1,000,000,000-10,000,000,000) of a fully virulent strain of tularemia bacteria rabbits usually die in a short time, on the average two-five days after infection, and only in a few cases does this time drag on to 10-12 days. After infection with a dose of 100,000,000 microbes the rabbits usually die after three-six days, but occasional animals can die later, up to the 10th day. We know of rare cases of death in rabbits even 12-24 hours after infection with a dose of 10,000,000,000 microbes.

The comparatively short survival time of rabbits after injection with massive doses of the pathogen as well as the absence of necrotic nodules in the organs in such cases indicate the predominance of toxicosis as the cause of death. This phenomenon is similar to what is observed in white rats. In rabbits dying of tularemia in a short time (one-four days) a very marked hyperemia is noted of the blood vessels of the subcutaneous tissue, hemorrhage into the lymph nodes, enlargement and densification of the spleen and liver. In the case of longer survival times hyperemia of the subcutaneous blood vessels is less marked; the reaction of the regional lymph nodes on the side of the infection is greater and is expressed in an enlargement and densification of them, and in some cases the formation of areas of necrosis. The spleen, liver, lungs can have disseminated necrotic nodules. Histologic changes found in the organs of rabbits dying of tularemia are similar to those in guinea pigs (V. N. Kartashova, 1935; S. V. Kagramanov, 1943).

A culture from the organs of rabbits dying even on the third-fifth day can remain sterile and for the purpose of isolating the culture it is necessary to resort to biological tests. According to the data of T. N. Dunayeva, on bacteriological examination of the organs of 13 rabbits which died of tularemia cultures from the regional lymph nodes were positive in nine rabbits; of the spleen, in seven; of the liver, in three; of the lung, in two; of the blood, in two. In a number of cases the growth was sparse, and appeared between the third and ninth days of incubation. In a rabbit which died on the seventh day after in-



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fection with a dose of 10,000,000,000 microbes it was determined by titration on white mice that the seeding of the spleen was with 100,000 tularemia bacteria per gram of tissue. Bacterioscopy of the organs of animals dying of tularemia usually is negative, which is in agreement with the relatively moderate seeding of organs and tissues of the rabbit even at the time of death.

In rabbits dying in a period of less than a day after subcutaneous infection with a massive dose of a virulent strain bacterioscopy of the smears from the organs (spleen, lymph node) may be positive in a number of cases, including bacteria which can be found in blood smears in some rabbits (N. G. Olsuf'yev and Ye. N. Tolstukhina, 1949). The bacteriology of tularemia in rabbits after subcutaneous infection with a lethal dose (the dose was not indicated by the authors) has been studied by I. S. Tinker and M. S. Drozhevskina (1948). However, for the purpose of detecting the bacteria and determining their numbers they used an inadequately sensitive method of culture on liquid egg yolk medium; therefore, the rate of spread of the bacteria in the rabbit organism after infection with a lethal dose as well as the quantitative indices of seeding of the tissues at different stages of the development of the infectious process are apparently too low.

V. P. Dzhanpoladova (1947) points out that after subcutaneous infection of rabbits with doses of 10 to 100,000 microbes of a virulent strain leukocytosis is noted in the animals with a benign course of the disease (two-four-and-a-half times greater than normal). In some rabbits it reached the maximum on the fourth day after infection; in others, on the 10th-12th day; in one rabbit, only on the 19th day. The white blood count gradually returned to normal, usually on the 12th-17th day. In two severely sick rabbits and in one which died leukopenia was noted. In a later work (V. P. Dzhanpoladova, 1951) it was pointed out that leukocytosis in rabbits is the result of an increase in the absolute pseudoeosinophil and lymphocyte counts with the absence or small numbers of eosinophils.

Characteristics of Tularemia after Other Methods of Infection. Rabbits are almost as susceptible to aspiration infection as to subcutaneous. For example, in the experiments of N. G. Olsuf'yev and O. S. Yemel'yanova, after a very fine spray of a suspension of a virulent tularemia strain, 503 (in the form of a mist) in a chamber, aspiration infection of the rabbits was obtained from doses totalling several microbes (from three to six) according to the CKI optical standard. Higher doses caused infection in rabbits in all cases also. With small doses the rabbits easily became sick and rapidly recovered, but with increase in the dose the severity of the process increased, and after the aspiration of 60,000 microbes one of the rabbits died on the

13th day with signs of a total tularemic pneumonia. In the rabbits which recovered necrotic changes were found in the lungs; the intensity of them to some degree was proportional to the infective dose. In the blood of all rabbits which recovered there were agglutinins to *F. tularensis*. These experiments showed that the superimposition of intercurrent infections on the tularemic process because of the presence of commensal microbes in the respiratory tract (for example, *Pasteurella*) can markedly alter the course of the disease, making it more severe and with a fatal outcome even after infection with a relatively small dose.

Experiments of conjunctival and intraocular infection of rabbits were performed by O. A. Dudinov (1940). (D. A. Golov prepared the culture for infection and generally observed the course of the experiments). The animals were injected with relatively large doses which, however, were inadequately standardized, because the number of bacteria introduced was calculated per portion of the culture taken up from the coagulated egg yolk medium with a platinum loop. In a number of experiments the rabbits were infected with 1/10 loop (which probably amounts to no less than 10,000,000 microbes by the GKI standard); in others, with 1/100 of a loop. In all cases, the rabbits became sick and part of the animals died of tularemia. After conjunctival infection edema of the lids, nodular lesions of the conjunctivae were observed which later changed into necrotic foci located on the retrotarsal fold, copious purulent excretion during the first week and sparser excretion in the subsequent period. The eyes began to suppurate on the 14th-18th day of the disease. The resolution of the inflammatory changes in the eyes was completed in the fourth-fifth week in cases in which the rabbits recovered. A biological examination (biological tests on mice) of the excretions of the affected eye showed *F. tularensis* continuously until 20 days after the infection. From the 20th through the 24th day only one examination out of four was positive, which was evidence of the fact that tularemia bacteria had been completely eliminated from the eye. After the 24th day the observations were stopped. After the introduction of a bacterial suspension into the cornea the involvement of the eye developed in a manner similar to interstitial keratitis localized to the area of infection. After healing an inconstant leukoma was noted. The rabbits became most severely ill after the intraocular method of infection. Part of the animals died of tularemia on the fifth-11th days; the others were killed. Intraocular infection was associated with a severe deformity of the eye up to the point of rupture of the eyeball.

Rabbits show much greater sensitivity to intravenous infection than to subcutaneous. In the experiments of L. M. Khatenever

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(according to the archive data of the tularemia laboratory), the MLC<sub>50</sub> on intravenous injection of a virulent culture into rabbits was equal to 10,000,000 microbes; on subcutaneous injection, 1,000,000,000. In the experiments of the same author with another strain the rabbits died after the intravenous injection of doses of 10,000 and 100,000 microbes and survived after subcutaneous infection with doses of 100,000,000 and 1,000,000,000 microbes (L. M. Khatenever, 1943). These data clearly show the presence of barriers to the passage of bacteria into the internal organs from the subcutaneous tissue in which they are injected. According to the data of O. S. Yemel'yanova a dose of 1,000 microbes of fully virulent strains on intravenous injection is not lethal to the rabbit. In the third week high agglutination titers are found in them. The author considers this method of infection convenient for obtaining agglutinating ViO sera (see Chapter III).

Immunity. Recovery from disease caused by infection with sublethal doses of tularemia microbe confers a quite strong immunity against repeated infection on the rabbits (subcutaneous, intradermal infection, etc.) with several lethal doses of the virulent strain (L. M. Khatenever, V. P. Dzhanpoladova). If the dose injected is not excessively large with respect to the number of microbes contained in it, the rabbits usually react to a second infection only with a slight and brief temperature elevation as well as a limited local reaction. According to the data of V. P. Dzhanpoladova (1949), immunity in rabbits which have had tularemia is first nonsterile but then becomes sterile and can be retained for three years, which is not the limit. The author points out that rabbits in various cases can remain bacterial carriers up to one year.

The elimination of bacteria from the bodies of the majority of rabbits which recover is apparently completed at the same time as in white rats, that is, usually between the 30th and 40th day after infection. In one of the experiments of L. M. Khatenever 30-60 days after infection the pathogen was found in the organs (by the biological method) in only four out of 10 rabbits which recovered from tularemia. In an experiment with aspiration infection, on an examination made on the 20th day after inhalation, tularemia microbes were found in all four rabbits used, and after 30 days this result was obtained in only two rabbits out of the four which recovered (N. G. Olsuf'yev and O. S. Yemel'yanova). In the experiment of subcutaneous infection with doses of 1,000 to 100,000,000 microbes of strain 503 bacterial carriage was found in four out of eight rabbits which recovered after 24-30 days (O. S. Yemel'yanova). In another experiment, with a similar infection of the rabbits using doses of 10,000,000-1,000,000,000 microbes of fully virulent strains bacteria were found after 60 days in only one

of the 13 animals which recovered (O. S. Yemel'yanova and T. N. Dunayeva).

During the course of development of the infection (when it has a benign course) an allergic reorganization of the body occurs in the rabbits which is readily detectable by the intradermal tularin test; also, active production of antibodies is noted which are found in the agglutination, precipitation and complement-fixation tests. It is also possible to show an increase in phagocyte activity, as demonstrated by means of performance of the opsonocytophagic test. In nonimmune rabbits antibodies to tularemia bacteria are entirely absent.

For the purpose of studying the development of the immunity reaction rabbits together with guinea pigs constitute exceptionally convenient laboratory models. With minimum doses of subcutaneous or intradermal infection (1-10-100 microbes) of a virulent culture agglutinins begin to be found in the blood of rabbits after the 10th day from the beginning of the experiment, and on the 20th day the serum titers can reach 1:1280-1:2560, but even on the 30th day they drop, on the average, by two times (T. N. Dunayeva). Approximately the same figures have been noted in the experiments of V. P. Dzhaneladova (1948). With massive doses of subcutaneous infection of a virulent tularemia culture agglutinins begin to be found in the rabbits on the sixth-seventh day and sometimes on the fourth-fifth day and reach a maximum on the 12th-14th day (L. M. Khatenever, 1943; O. S. Yemel'yanova). The serum titers usually reach 1:640-1:1280; less often, 1:2560. Reduction of them is noted after the 20th-25th day, but even after two months they can amount to 1:400, on the average. Antibody production in the rabbits is therefore subordinate to the same rules and regulations as in white rats. In other experiments, using subcutaneous infection with sublethal doses, L. M. Khatenever established the fact that agglutinins are found in the rabbits up to two years (the observation period was indefinitely long), but the serum titers were, on the average, 1:20.

The data of A. V. Mashkov (1946) are interesting; he used a modified (more sensitive) method of agglutination and found antibodies in rabbits beginning with the third-fourth day after infection with a virulent strain (the dose is not indicated). In the third week the serum titers reached 1:1600-1:5000; later, they began to decrease. According to the data of the same author the skin allergic reactivity of rabbits infected with a sublethal dose of a virulent strain is found after the sixth-seventh day (by the injection of the usual dose of tularin).

In rabbits which have recovered from tularemia V. P. Dzhaneladova (1948) found allergic reactivity for a year (the observation period), whereby six months after infection the reaction at the

injection site of tularin was weaker than after a month. The experience of our laboratory shows that for the purpose of detecting skin allergic reactivity in rabbits it is best to use tularin containing 1,000,000,000 microbes per cc, that is, to inject a dose of allergen intradermally 10 times greater than is usually used in medical practice. B. Ya. El'bert and N. A. Gaytskiy (1941) used a quintuple dose of allergen in rabbits for these purposes (50,000,000 microbes were injected in 0.1 cc).

In the sera of rabbits which recovered from tularemia V. P. Dzhanpoladova (1948) detected precipitins. Usually, the sera reacted only with undiluted antigen. The precipitins were found in the rabbits for nine months after infection, and in one rabbit, after 12 months, but chiefly in those cases where the agglutination titer of the sera being studied was 1:100 or higher. The same author found complement-fixing substances in the sera of rabbits in the first four months after their recovery. V. P. Dzhanpoladova (1949) describes phagocytosis in rabbits, which she observed in vitro by performing the opsonocytophagic test, and in vivo by studying blood smears of animals which recovered. In the latter case the author showed phagocytized bacteria in "polymorphonuclear neutrophilic leukocytes and less often, in monocytes". Phagocytosis was readily found in rabbits beginning with the 14th day until the 30th day after infection; in every field there were three-seven phagocytes and in every leukocyte there were 30-60 bacteria, "but sometimes so many it was impossible to count". In another work (1947) Dzhanpoladova reported that on the examination of smears of two rabbits killed four months after infection (the dose and the strain are not mentioned) she found a large number of phagocytized bacteria in the spleen -- in macrophages; and in the liver, in the Kupffer cells. In every field there were three to five phagocytes "packed with tularemia bacteria". A negative result of cultures from both rabbits as well as failure of biological examination of the organs of one of them cause us to doubt that the author actually observed phagocytosis rather than degenerative granulation of cells. Similarly, we have reason to doubt a number of other observations by the author on phagocytosis in tularemia of rabbits in view of the fact that these investigations were made with the use of selective staining of tularemia bacteria (Romanowsky-Giemsa staining used by the author is inadequate for these purposes) and were not accompanied by bacteriological studies which proved them.

#### **Rules and Regulations of Pathogenesis of Experimental Tularemia**

The data presented on experimental tularemia in laboratory

animals permit making certain generalizations, despite the partial gaps in the study of it in various species, particularly in rabbits.

Of the species of animals analyzed white mice are most susceptible and most sensitive to tularemia. The sensitivity of guinea pigs to tularemia is somewhat less than that of white mice, although the end result of the infection with a fully virulent strain is the same -- death. The difference lies in the fact that the guinea pigs die after a longer time, and the seeding of the internal organs and tissues and particularly of the blood in guinea pigs is less at the time of death than in white mice. This indicates the fact that the infectious process in guinea pigs is partially inhibited by the natural defensive forces of the body, whereas in white mice the body is practically defenseless against invasion and multiplication of tularemia bacteria. White rats and rabbits are very much different from the highly sensitive white mice and guinea pigs in their low degree of sensitivity to tularemia (the difference is approximately 100,000,000 or 1,000,000,000 times), whereas they are similar to each other in this characteristic. Rabbits are somewhat more resistant, which is seen from a determination of the M.I.C.D. The different relations of laboratory animals to tularemia infection is a species characteristic.

In spite of the dissimilarity in the infectious sensitivity of the guinea pig and white mice, on the one hand, from that of the white rat and rabbit, on the other, the degree of their susceptibility to tularemia infection (that is, the ability to become infected) is very close, and in a number of cases is the same: any of the species which we are analyzing can, in practice, be infected by the subcutaneous or intradermal injection of a dose of one microbe according to the GKI optical standard.

Study of the infectious process in various species of animals makes it possible to divide it, based chiefly on bacteriological characteristics, into the following phases: I -- adaptation; II -- regional infection; III -- hematogenous dissemination and focal spread of the infection; IV -- septicemia, and in case of recovery -- IVa -- subsidence of the infection (Fig 23). This schema is the same as that of P. F. Zdrodovskiy, which he proposed for brucellosis, with the difference only that we have subdivided the phase of generalized infection into two phases: a) hematogenous dissemination and focal spread of the infection; b) septicemia. Clinical observations give us the basis for subdividing the phases of hematogenous dissemination and focal spread of the infection into its initial part, as bacteriemic (toxemic) and its subsequent part, as being allergic (see Chapter VII). A more thorough study of the pathogenesis of tularemia in laboratory animals will probably also permit confirmation of this division. The occurrence



of the allergic phase may be evidenced by a positive skin test with an allergen.

Our subdivisions of the infectious process to a certain degree coincide with the schema of I. N. Mayskiy (1949, 1953) which he developed mainly as applied to tularemia in man. He distinguished the following phases: 1. Adaptation; 2. Lymphatic defense reaction; 3. Bacteriemia; 4. Focal lesions; 5. Convalescence. This schema does not stand up nomenclature-wise; for example, in some cases the names of the phases are given on the basis of a bacteriological characterization of the process (adaptation phase and phase of bacteriemia); in others, based on pathophysiological and pathological characteristics (phases of lymphatic defensive reactions, focal lesions); in still others, on clinical characteristics (convalescence). There is no phase of septicemia, the presence of which is obligatory in an infectious process occurring acutely with a fatal outcome, characteristic of guinea pigs and other animals highly sensitive to tularemia. An acute course of tularemia similar to septicemia with a fatal outcome is known in people also, particularly in the United States. The phase of septicemia is not the equivalent of I. N. Mayskiy's phase of bacteriemia, which is better designated the phase of hematogenous dissemination and focal spread of the infection, which better depicts its nature.

I. S. Tinker and M. S. Drozhevskina (1943), on the basis of a study of tularemia in guinea pigs, white mice and rabbits, distinguished the following phases: 1) production of the initial effects; 2) the primary complex; 3) hematogenous dissemination; 4) a generalized process; 5) septicemia. In the schema of these authors phases 1, 2, 3, and 5 correspond to our phases I, II, III and IV; only the names are different. However, in this case we do not see any reason for distinguishing specially phase 4 and considering it only a phase of a generalized process, since the phase of hematogenous dissemination and the phase of septicemia following it represent only different stages of generalization of the infection. The phase of subsidence of the infection in cases of recovery has not been provided for in this schema.

Finally, we should like to mention the idea of Kh. Kh. Planel'yev and S. L. Krasinskaya (1950), who studied chiefly the initial stages of the pathogenesis of the Breslau variety of *S. paratyphi* in white mice, that the adaptation phase be named the "cryptobiotic" phase, and the phase of regional infection be called the "lymphatic" phase. However, we consider these names less apt than those adopted in P. F. Zdrodovskiy's schema.

The phase of adaptation after intradermal injection of single tularemia bacteria can last three days. Bacteriological and histological analysis of this phase shows that the initial stages of bacteri-

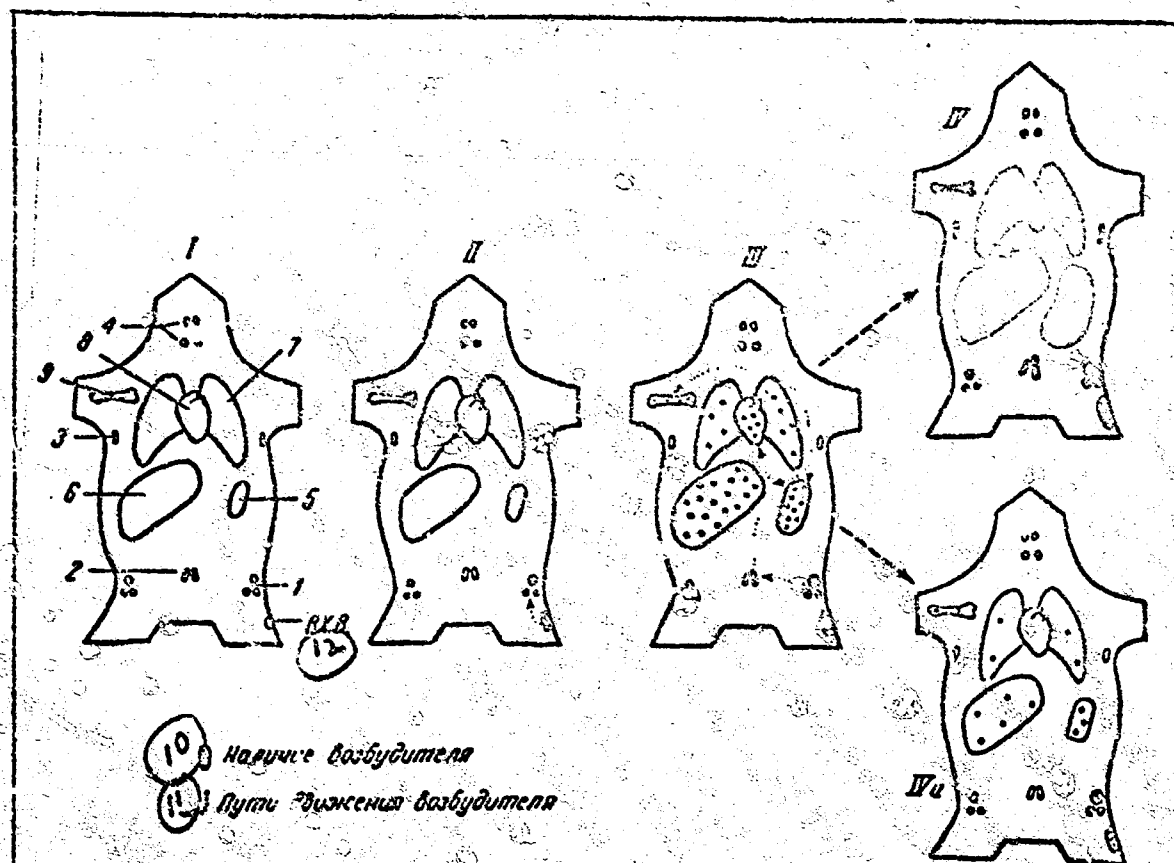


Fig 23. Phases of the Infectious Process in Experimental Tularemia. Schema. I. Adaptation; II. Regional infection; III. Hematogenous dissemination and focal spread of the infection; IV. Septicemia; IVa. Subsidence of the infection. 1. Inguinal lymph nodes; 2. Retroperitoneal lymph nodes; 3. Axillary lymph node; 4. Submaxillary and cervical lymph nodes; 5. Spleen; 6. Liver; 7. Lung; 8. Heart; 9. Bone marrow; 10. Presence of the pathogen; 11. Routes of movement of the pathogen; 12. Porta's of entry of the infection.

multiplication in the skin at the injection site are accompanied by inflammatory reactions. Therefore, in tularemia the primary focus of infection, no matter how miniature it might be, develops in the tissue at the site of incorporation of the pathogen, whereas the focus which arises in the regional lymph node after penetration of bacteria there is secondary.

The phase of regional infection occupies a special place in the pathogenesis of tularemia. By multiplying in the tissues of the lymph node (or nodes) the pathogen secures the opportunity of further spread in the body on a higher quantitative level than during the adapta-



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tion phase. This is a distinctive "springboard" for the infection. I. N. Mayskiy, I. S. Tinker and others regard the lymphatic system as a barrier to tularemia bacteria which the latter must overcome. Hence, we have the suggestion by I. N. Mayskiy that it be called the "phase of lymphatic defensive reactions of the body". Kh. Kh. Planel'yes (1950), on the basis of investigations of the staphylococcal and paratyphoid infection of white mice and pneumococcal infection of white rats in combination with his co-workers, believes that the lymph nodes serve as a favorable place for settlement of bacteria, where they multiply relatively freely, forming the primary focus of infection. Penetration of tularemia bacteria into the lymphatic system and further movement through it into the body are explained by some authors (I. S. Tinker and M. S. Drozhevskina, 1948 and others) by "lymphotropism" of the pathogen. However, other small particles, for example, of India ink or graphite, if injected into the tissue, also enter the lymphatics because of the structural characteristics of the walls of the latter, and are carried with the lymph flow into the lymph nodes (D. A. Zhdanov, 1952). We cannot speak of a "lymphotropism" of such particles.

According to our data, in white mice, guinea pigs and white rats tularemia bacteria multiply in the tissue of the regional lymph node, without encountering any particular resistance on the part of the macroorganism. If the resistance of the guinea pig organism to tularemia is increased, for example, by means of immunization with attenuated strains, the barrier function of the regional lymph node as well as of the tissues at the injection site becomes fully expressed in this animal (R. A. Savel'yeva and A. P. Gindin).

The transition of the regional infection into the phase of hematogenous dissemination (focal spread) occurred of necessity in all the species of laboratory animals which we analyzed regardless of the method or dose of infection. With the onset of bacteriemia the disease passes into the clinically overt period from the incubation period.

Exudative phenomena, on which proliferation of reticulo-endothelial cells is rapidly superimposed and in which the formation of specific granulomas similar to those in tuberculosis, brucellosis and other infectious diseases at sites of settlement and multiplication of bacteria occur, are characteristic of the inflammatory responses of the macroorganism in tularemia. In the initial phases of development of the infection the inflammatory changes are of a local character, but during the phase of hematogenous dissemination the granulomatous process includes the internal organs in proportion to their degree of bacterial seeding (R. A. Savel'yeva and A. P. Gindin). In this phase, in different species of animals the process differs substantially in

accordance with the degree of their infectious sensitivity (resp. resistance) to tularemia and the level of seeding of the internal organs.

In white mice and guinea pigs a high degree of sensitivity of the reticuloendothelial cells and other tissues to the effect of tularemia bacteria on them leads to the fact that penetration of the microbes into the internal organs of the animals is accompanied by multiple severe (necrotic) injuries of the tissues with a functional disorder in the vital organs (bone marrow, suprarenal glands, spleen, liver, etc.), which completely suppresses immunogenesis. Throughout the infectious process in the animals there are no specific immunological reactions. Seeding of the organs with bacteria increases through the enlargement of the foci and metastasis of them; injury to the tissues is intensified, and the process goes into the phase of septicemia ending in death.

In white rats and rabbits, as the result of the lower sensitivity of tissues to the effect of tularemia bacteria, the inflammatory processes are of a more localized and benign character, without suppressing immunogenesis. In rats, as a rule, the granulomas do not become necrotic in the internal organs, but the antibodies begin to be found on the sixth-seventh day after infection when the agglutination test is performed in the usual way. The acquired immunity which arises limits the further spread of the bacteria in the vital organs with its development and, without going into the phase of septicemia the infection begins to subside. At the time of recovery the immunological indices (antibodies; in rabbits, allergy also) reach their maximum degrees of expression. B. Ya. El'bert and N. A. Gayskiy (1941) point out that the immune serum of rabbits which recovered from tularemia possesses the ability to neutralize the necrotic effect produced by *F. tularensis*. Subsequently, under the influence of the high-strength immunity which develops the elimination of bacteria from the body and the recovery of the injured tissues are completed quite quickly (usually, between the 30th and 40th day after infection), and the immunity goes into the sterile phase, remaining for a long time. Occasional exceptions do not change the general rules and regulations. The last to be free of bacteria are usually the regional lymph nodes.

Inflammatory changes in the animal tissues (exudative processes, granuloma formation, etc.) occurring under the influence of living or killed virulent bacteria undoubtedly are associated with an endotoxin liberated from destruction of microbes by cells and juices of the macroorganism.

After the injection of large doses of the pathogen the rapid death of animals which are not very sensitive to tularemia -- white rats and rabbits -- with the picture of acute toxicosis and a relatively

low degree of seeding of the organs with bacteria clearly indicates the presence of toxic substances in the tularemia bacteria. However, these substances, like the rickettsial toxin, for example, are different in their properties from ordinary bacterial endotoxins, which makes study of them difficult.

Judging from the data of study of the antigenic structure of tularemia bacteria, the toxin is present in their capsular portion and associated with Vi antigenic substances (N. G. Olsuf'yev and O. S. Yemel'yanova, 1957).

All the species of laboratory animals analyzed show susceptibility to subcutaneous, intradermal and aspiration infections almost equally. With these methods of administration of the bacteria the minimum infective dose is almost the same or practically the same. The conjunctival method of infection is notably less effective, and the alimentary method is in last place. The application of the bacteria to the intact skin causes infection only after the use of a large dose, but scarification of the skin increases the possibility of penetration of the bacteria.

Each of the infection methods has its own characteristic localization of the primary lesions, which can be determined usually without difficulty by the degree of expression of the inflammatory changes in the tissues at the site of incorporation of bacteria and the degree of involvement of the regional lymph nodes. These changes are particularly demonstrative in guinea pigs. The results of experiments of aspiration infection of guinea pigs and rabbits confirmed G. P. Rudnev's conception of the primary pneumonic form of tularemia. For animals which are not very sensitive this form should be considered the most severe; a fatal outcome can be observed from doses which on injection through the skin produce only a benign course of the disease. However, administration of minimum doses of the pathogen by the aspiration route can cause a mild disease in slightly sensitive animals also as the result of localization of the focus in the lung.

In tularemia the relationship between the infective dose and the rate of development of the infectious process is established in a quite clear-cut manner. In white mice and guinea pigs, with increase in the dose, the survival time is shortened, whereby in mice the seeding of the organs and tissues with bacteria is high at the time of death regardless of increase in the dose, whereas in guinea pigs the seeding of the organ increases with the dose. In white rats and rabbits, with increase in the dose injected, the clinical expression of the process increases, which is particularly well expressed in weight changes. Large doses cause a fatal outcome, and the time needed for it to occur is shorter the higher the dose. However, in these animals individual

characteristics of the organism can have a marked influence, in one direction or another, on the development of the infectious process and on its outcome. Therefore, the experiment shows precisely that a great range of differences in the severity of the course of tularemia infection, localization of primary lesions, etc. can be achieved through infection with a microbe of the same virulence. These differences show a distinct relationship to the infective dose, the route of penetration of the pathogen and partly, the individual characteristics of the macroorganism. In this connection, experimental data on laboratory animals are in good agreement with epidemiological and clinical observations.

In dealing with problems of bacteriological diagnosis of tularemia in laboratory animals, it should be noted that the most sensitive method of detecting tularemia bacteria is the biological test method with the use of the white mouse for the passage. It makes it possible to show even single viable virulent bacteria without error in the infected tissues of any species of animal. The method of culture on synthetic nutrient media usually used, for example, egg yolk, by making an impression or rubbing in a piece of tissue, is much less effective than the biological method. According to the summarized data of T. N. Dunayeva's investigation of organs of different animals it may be concluded that with a content of 100-1,000 bacteria (as shown by the method of titration on white mice) per gram of investigated splenic or lymph node tissue only 12 percent of cultures on coagulated egg yolk medium are positive; a result close to 100 percent in the cultures was noted only when tissues contained 1,000,000-10,000,000 bacteria or more per gram (Table 15).

The use of liquid yolk medium for cultures does not essentially change the result, which may be judged by the investigations of I. S. Tinker and M. S. Drozhevskina. However, the use of a culture of an organ suspension on petri dishes containing blood media is practically the equal in sensitivity to the biological method of investigation. In last place with regard to sensitivity is bacterioscopy and the thermoprecipitation reaction. Experiments on guinea pigs, white mice and white rats indicate that tularemia bacteria can be found reliably by these methods only in those organs in which the concentration of microbes exceeds 1,000,000,000 per gram of tissue.

In accordance with what has been stated the method of bacterioscopy (or precipitation) can be used successfully for the diagnosis of tularemia chiefly in investigation of organs of white mice dying of tularemia. The method of culture on nutrient media is applicable to investigation of organs of animals of any species dying of tularemia, but cultures from rabbits and white rats cannot always give a positive

Table 15

The Degree to which *P. Tularensis* can be Plated Out of Organs (Lymph Nodes and Spleen) of Killed Animals as a Function of the Intensity of Seeding per Gram of Tissue (after T. M. Danayeva)

Вид животного	Количество бактерий на 1 г ткани											
	100-1000			10 000-100 000			1-10 млн			100 млн.		
	Результаты посевов											
	всего исследованных	из них положительных	сроки появления роста (сутки)	всего исследованных	из них положительных	сроки появления роста (сутки)	всего исследованных	из них положительных	сроки появления роста (сутки)	всего исследованных	из них положительных	сроки появления роста (сутки)
Белая мышь . . .	17	2	6-7	2	1	5	3	3	2-3	4	4	1
Морская свинка	5	0	—	3	2	2-4	11	9	2-4	6	6	1-3
Белая крыса . .	11	2	6-7	13	5	4-6	5	2	2-5	—	—	—
Всего . . .	33	4	6-7	18	8	2-6	19	14	2-5	10	10	1-3
	12,1%			44,4%			73,6%			100%		

Note: Culture made by impressing a piece of tissue on coagulated yolk medium. 1. Species of animal; 2. No. of bacteria per gram of tissue; 3. Results of culture; 4. Total investigations; 5. Of these, number positive; 6. Time of appearance of growth (days); 7. Million; 8. White mouse; 9. Guinea pig; 10. White rat; 11. Total.

result because of the inadequate seeding of the animal tissues with tularemia bacteria. Finally, the biological method is used in those cases where the other methods may be ineffective.

#### Conclusion

Data accumulated on experimental tularemia in laboratory animals permit a quite complete evaluation of various species as objects to be used for biological test for diagnostic purposes as well as in the capacity of models in the study of various problems of pathology, immunology, etc. Although a number of details of the infectious process (particularly with methods of infection other than subcutaneous) has been inadequately studied in various species of animals, the general picture of the pathogenesis of tularemia has been quite fully defin-

eated at the present time. We have in mind the bacteriology and pathology of the infection but not the pathophysiology, which has practically not been studied in tularemia. The first and very interesting investigations along this line were made by A. G. Kratinov and co-workers but, unfortunately, they were not further developed. It is to be hoped that there will be investigators of problems of the pathophysiology of experimental tularemia with extensive use of biochemistry for this purpose in the very near future.

## Chapter VI

### The Epidemiology of Tularemia

(G. P. Ayrapet'yan participated in selecting material for this Chapter)

#### General Comments

Problems of the epidemiology of tularemia have attracted the attention of Soviet investigators since the first recognized outbreak of this disease near Astrakhan' in 1926 (S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova, 1928). A great contribution to the study of the epidemiology of tularemia was made by A. A. Vol'ferts, D. A. Golov, L. M. Khatenever, G. Ya. Sinay, P. V. Somov, S. P. Karpov, G. P. Rudnev, K. F. Akinfiyev, L. V. Gromashevskiy, I. I. Yelkin, A. A. Maksimov, Yu. A. Myasnikov, M. F. Shumeter and others. These investigations showed distinctly the difference between the conditions under which people are infected with tularemia in the USSR and in foreign countries, particularly in the United States. While in the United States the main source of the infection in nature is considered to be hares, and people are most often infected from these animals in removing the pelts, dressing the carcass or consuming it as food (see Chapter II), in the USSR the main sources of infection are water rats and small mouse-like rodents, from which people are infected under the most diverse conditions.

In studying the various outbreaks of tularemia, carefully analyzing the conditions under which they occur and develop, and taking antiepidemic measures with the aim of suppressing these outbreaks, Soviet investigators have accumulated valuable material on the epidemiology of tularemia making it possible to determine the main rules and regulations of the epidemic process in this infection and outline effective methods of controlling it. A particularly difficult test was given to Soviet epidemiologists during the years of the Second World War, when under conditions of the situation at the front as well as on territories liberated from enemy occupation large outbreaks of tularemia of an unusual epidemiology were encountered and epidemiologists had to take extensive measures for eliminating them. Subsequently, a very important part was played by a clarification of the various epidemiological types of disease (outbreaks) and their classification as well as the determination of their connection with various types of natural foci, which made it possible to carry out the necessary preventive measures.



differentiated manner.

The study of these problems can hardly be considered entirely completed. In the future new facts may be established which extend current ideas of the lines of circulation of the infection and the conditions under which people become infected with tularemia.

In the present Chapter the rules and regulations of the epidemic process are being analyzed, that is, the causes and conditions of occurrence, spread and arrest of cases of tularemia among people, whereas problems of prophylaxis are presented in Chapters X and XI. Thereby, we shall characterize the features not only of the epidemic outbreaks but also of the sporadic cases. Such an approach is necessary at least because at present, as the result of broad prophylactic measures taken, tularemia is usually noted only in the form of isolated cases, whereas outbreaks have become a rare phenomenon, and in the very near future public health organs will be confronted with the task of eliminating them completely. In accordance with what has been stated, the epidemiological classification which we have adopted in this Chapter analyzes the types of cases of tularemia, including both outbreaks and sporadic cases.

#### **Epidemiological Characteristics of Tularemia and Classification of Types of the Disease (Outbreaks)**

In an epidemiological respect tularemia is defined as a zoonosis which has a natural focalization maintained basically by wild rodents and blood-sucking arthropods. Just as in the case of the epizootology of tularemia (see Chapter V), not only a multitude of sources of infection and routes of transmission but also a considerable variety of conditions under which infection is possible are characteristic of the epidemiology of this infectious disease. One of the main characteristics of the epidemiology of tularemia is the fact that the disease occurs in people in rural localities, which is associated with the natural focalization of this infectious disease and the absence of conditions for its spread in the large cities among domestic rodents. Thereby tularemia is different from a number of other diseases with natural foci such as plague, listerellosis, pseudotuberculosis and others capable of spreading in the population of city rodents with subsequent infection of people. Cases in which people are infected with tularemia under city conditions, particularly a large city, are rare and are associated with importation of infected food products or animals from a rural locality. Cases in city dwellers usually occur because of infection of them from a temporary trip to a rural locality, where natural foci of tularemia exist.



On the territory of the USSR the most frequent sources of infection accounting for infection of people are common vole, house mice and water rats; to a lesser degree, hares, muskrats, hamsters and some other rodents. As has been pointed out in Chapter V, these species of animals are widespread, become acutely sick with tularemia with massive dissemination of tularemia bacteria in the organs and tissues (group I) and are affected by tularemia epizootics in large numbers. Of the blood-sucking arthropods the blood-sucking Diptera (mosquitoes, horseflies) and ixodid ticks are of the greatest epidemiological significance as vectors of the infection to man.

A characteristic feature of the tularemia microbe is its relative resistance to environmental conditions. The pathogen of tularemia can be preserved in water, milk, frozen meat, the bodies of rodents which die of tularemia, in grain, straw, etc. (see Chapter III) for a long time, particularly with a low environmental temperature, which makes its impression on the epidemiology of tularemia. The microbe is characterized also by the fact that it is capable of penetrating into the human and animal body through minor scratches on the skin and through the intact mucosae of the eyes, mouth, and pharynx (particularly through the tonsils), gastrointestinal tract and respiratory tract, and in the case of a massive dose of the infection the microbes can penetrate through the intact skin also (see Chapter IV, page 110). The localization of the primary lesions (local focus of inflammation as well as inflammation of the regional lymph nodes) usually clearly indicates the routes of penetration of tularemia bacteria into the body, which makes it possible to draw clinical-epidemiological parallels (G. P. Rudnev and others).

The following specific methods (mechanisms) of infection of man with tularemia are distinguished: contact, alimentary, aspiration, and arthropod-borne. The last method is best called the inoculative method if we have in mind infection through the bite of a blood-sucking arthropod in contrast to contact (or contaminative) from crushing the vector on the skin surface; the term "transmissive" (which is generally used in the Russian literature to mean arthropod-borne) should be used for designating the transmission factor and the type of disease (of outbreaks).

Contact infection through the skin and the external mucosae represents a frequent route of infection of people with tularemia. The infection can occur as the result of direct contact of man with animals sick with (or which have died from) tularemia, particularly in skinning or dressing the carcasses, through the bites of animals, as well as through contact with substrates contaminated by the excretions of sick animals (water, grain, straw, etc.). Infection through the external

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mucosae, particularly through the conjunctiva of the eye, can occur under the same conditions in the case of mechanical transfer of the pathogen by contaminated hands.

Infection by the aspiration route often occurs in people occupied in tilling cereal crops (threshing, working on the winnowing machine, etc.) and fruits as well as in the transportation of straw, hay and other objects contaminated with the excretions of rodents sick with tularemia.

Infection by the alimentary route occurs from the consumption of infected fruit products or utilization of infected water for drinking.

Infection by vectors (blood-sucking arthropods) usually occurs by means of a puncture in the human skin with the proboscis of arthropods and injection (inoculation) of the tularemia pathogen into the wound, but it can also be observed after crushing an arthropod on the skin surface or after it enters the eye.

One of the characteristic epidemiological features of tularemia is an almost 100% susceptibility of man to it without any differences in age as well as the fact that sick people are not contagious to healthy persons. The latter is explained in the features of the infectious process, which in man is characterized by relatively sparse seeding of the organs and tissues with tularemia bacteria, as the result of which the patient's excretions are practically noninfective for those around under ordinary conditions of contact with them (G. Ya. Sinay, 1940). The relatively small number of tularemia bacteria in the organism of a sick person is indicated by the negative results of bacterioscopy of the pathological material and the difficulties of isolating the pathogen from it by direct culture, as the result of which usually recourse is had to the biological examination (see Chapters VII and VIII). Various authors believed that the absence of infections coming from a sick person is associated with a reduction of the virulence of the tularemia microbe in his body, but this opinion has been repudiated by the isolation of fully virulent cultures from sick people as well as by experiments on animals of groups II and III, the bodies of which are similar to the human body with respect to the degree of sensitivity to tularemia (see Chapter III).

The characteristics of tularemia infection mentioned above, that is, extensive adaptation of the tularemia pathogen in nature, the possibility of its transmission through animals or through various environmental objects (water, food and forage products, etc.) as well as the high degree of susceptibility of man to this infection have led to the fact that under certain conditions tularemia outbreaks have taken place on a grand scale with involvement of a considerable number of people.

Not uncommonly, tularemia outbreaks resemble an epidemic of influenza or malaria in character, and previously they frequently went under these diagnoses, which is evident by much material on the epidemiology of tularemia presented below. In some of the natural foci tularemia cases can be of a unique character, where for a long period of time the occurrence of disease is noted only among various persons or small groups of people. The nature and scale of tularemia epidemics depend on the rules and regulations created in the preepidemic and epidemic periods.

The course of the epidemic process depends on a number of conditions, including the characteristics of the natural focus itself, the composition of the reservoirs and vectors of the tularemia infection in the focus, the nature of the occupational and other activity of people, the population density, the size of the immune segment of the population, the intensity of contact between people and infected animals and environmental objects, the season and other factors. Depending on variations in the rodent census, particularly marked in the small representatives of this group of animals (voles, house mice, water rats), conditions for the occurrence of cases of tularemia in people are different in different years. At the present time, an important factor affecting reduction in the incidence of tularemia is vaccination of people being conducted in foci of this infectious disease in combination with other measures.

In the USSR epidemic outbreaks of tularemia associated with the water rat industry or with infection through blood-sucking Diptera (see Chapter I) first attracted attention and were studied in detail. In accordance with the conditions under which people became infected the name of "occupational" was reinforced for the first type of outbreak; the name of "arthropod-borne" gained prominence for the second. Subsequently, outbreaks were described with other infectious conditions, and they also obtained appropriate names. With the accumulation of data about epidemiological features of tularemia outbreaks or separate cases the need arose for a more precise definition and classification of them. The experience of foreign investigators in this respect was not very useful because of considerable differences in the epidemiology of tularemia in the Soviet Union and abroad as well as on account of the different approach of foreign investigators to the study of this problem. For example, in the United States tularemia is customarily divided into groups, basically in accordance with the sources of infection and its vectors, without the use of any special terms designating the epidemiological types of disease (see Chapter II).

Classification of the epidemiological types of cases of

tularemia (outbreaks) is very essential. It assists practising physicians and scientific research workers in becoming oriented to the epidemic process, making a more thorough study of epidemiological rules and regulations and developing measures not only for controlling the separate outbreaks but also for complete elimination of tularemia.

Problems of the epidemiological classification have been subjected to a special discussion in a number of publications of Soviet investigators (G. Ya. Sinay, 1944; G. Ya. Sinay and B. V. Voskresenskiy, 1943; G. P. Rudnev, 1943, 1950, 1959; I. N. Mayskiy, 1944, 1945, 1951; I. I. Yelkin, 1948, 1949, 1952; L. V. Gromashevskiy, 1948; L. M. Khatenever, 1948; Yu. A. Myasnikov and O. V. Ravdonikas, 1954; G. A. Kondrashkin, 1958 and others). Without going into all the details of the suggestions made, with which the reader can become acquainted from the original sources, we should like to note only that approaches of different authors to the solution of this problem were different. The majority of authors (G. Ya. Sinay, B. V. Voskresenskiy, I. N. Mayskiy, L. V. Gromashevskiy, L. M. Khatenever, G. P. Rudnev and others) expressed themselves in favor of the idea that epidemiological types of tularemia (outbreaks) be defined (classified) according to the infectious conditions and the transmission factors. In addition, the main sources of the infectious disease, mechanisms of infection, etc. were also pointed out. Based on the main principle of classification the authors considered the use of such names of the outbreaks as "industrial", "water", "agricultural", etc. justified. Other authors (Yu. A. Myasnikov and O. V. Ravdonikas, G. A. Kondrashkin) recommended making the sources of infection the basis of the epidemiological classification and correspondingly naming the types of cases (outbreaks) in accordance with the species of rodents: "vole-mouse", "water-vole", etc. (the author has in mind infection from water rats and muskrats). [Water voles are another name for water rats].

Finally, the suggestion was made (I. I. Yelkin) that the types of disease be classified by the mode of transmission (mechanism of infection) and that the epidemics be called appropriately "contact", "alimentary", etc. This latter as well as the previous classification are attractive by virtue of the fact that underlying them they have a single principle (mechanisms of infection or source of infection). However, they have essential defects because in these cases the leading factor in the epidemic process is not distinguished. Classification of epidemiological types of tularemia by sources of infection or mechanisms of infection cannot be considered correct if only because infection of people does not represent a purely biological phenomenon. It is most closely connected with human activity, that is, with the social factor. The proponents of the second and third principles of classifica-

tion simultaneously use the first principle, but as subordinate to the main principle proposed. For example, Yu. A. Myasnikov and O. V. Ravdonikas distinguish "domestic", "agricultural", "water", etc. types within the "vole-mouse" type. I. I. Yelkin distinguishes outbreaks of "water" and "food" origin, etc. within the limits of the "alimentary" type of epidemic.

In our further presentation we shall use the epidemiological classification based on the conditions of infection and the transmission factors, considering this principle most correct and best accepted by the majority of investigators. Naturally, in the characterization of one type of disease or another the sources of infection and mechanisms of infection are also included but as subordinate factors.

In all, at the present time nine epidemiological types of tularemia have been described, whereby for various types different variants can be distinguished depending on the differences in the sources of infection, the vectors or other conditions. In Table 23 a list of these types and their variants is presented with an indication of the main sources of infection or its vectors, mechanisms of infection, clinical forms, as well as types of natural foci with which various cases (outbreaks) are associated in their origin.

From the classification which we have adopted it is easy to see that the leading factor in the epidemic process is the one which actually determines the nature of spread of the infection, mechanisms of infection, etc., and, because of this, also the clinical picture of disease. Among the factors of this kind are agricultural operations, hunting, industrial processes, military operations, etc. True, along with this the following factors in the transmission of the infectious disease have been advanced as leading ones: water, arthropod, food products, etc. However, the group of such cases needs to be distinguished within the various types of outbreaks, since the characteristics of these transmission factors make a specific impression on the course of the epidemic, mechanisms of infection, clinical forms of disease and require special control measures.

The occurrence of a considerable portion of the various types of disease, for example, agricultural, domestic, food product, trench, water (infection through well water) and industrial (infection during processing agricultural products) is associated with small mouse-like rodents, as the result of which these diseases are frequently called "mouse" outbreaks.

In the classifications proposed previously various authors have distinguished tularemia in laboratory workers as a special type of disease. At the present time the need for distinguishing such a group has fallen away, because vaccination has completely eliminated tularemia

Table 23

## Epidemiological Types of Tularemia (Outbreaks) (after L. N. Maynskiy and N. G. Olsufiyev)

1. Характеристика эпидемического процесса (по локализации заболевания и географическому распространению)	2. Вспышки	3. Основные источники инфекции	4. Преобладающий механизм передачи	5. Преобладающий возбудитель	6. Тип источника инфекции и характерная локализация заболевания
7. Трансмиссивный	а) Зерновые crops и скот б) Зерновые crops и скот	а) Возвратная лихорадка б) Очаговые инфекции	12. Иксодовые клещи	13. Бубонная	а) Подково-базарный б) Сибирский, зуро-малый
8. Прямой		а) Возвратная лихорадка б) Очаговые инфекции	20. Контактный	22. Бубонная	24. Подково-базарный, сибирский, зуро-малый
9. Очаговый		Возвратная лихорадка, очаговые инфекции	21. Контактный	23. Антропо-бубонная, антропо-бубонная	25. Сибирский, зуро-малый, тифозный, меконный
10. Возвратная	а) Зерновые crops и скот б) Зерновые crops и скот	Возвратная лихорадка, очаговые инфекции	31. Контактный	33. Антропо-бубонная, антропо-бубонная	а) Препараторский, зооантропо-бубонный б) Зуро-малый, сибирский

1. Epidemiological type of disease (according to conditions of infection and transmission factors); 2. Variants; 3. Main sources of infection; 4. Predominant mechanism of infection; 5. Predominant form of disease; 6. Type of natural focus with which the given type of disease is most frequently associated; 7. Arthropod-borne; 8. Infection through mosquitoes and horseflies; 9. Infection through ixodid ticks; 10. Water rat, hares; 11. Common vole, hamsters, hares and other rodents; 12. Inoculative; 13. Ulcerative bubonic; 14. Soddy-alluvial-boggy; 15. Steppes, meadow;

(continued next page)

[continued from previous page]

field, forest; 16. Occupational; 17. Hunting-food; 18. Water rat, muskrat, hamster; 19. Hare; 20. Contact; 21. Contact, alimentary; 22. Bubonic, ulcerative-bubonic; 23. Anginal-bubonic, abdominal, ulcerative-bubonic; 24. Soddy-alluvial-boggy, steppe, and tugaic (tugai in the narrow sense of the word are forests near rivers; in the broader sense, the river valley type of locality in semidesert and desert zones); 25. Steppe, meadow-field, tugaic, forest; 26. Water; 27. Infection through water of brooks and other open water sources; 28. Infection through well water; 29. Water rat; 30. House mouse, common vole; 31. Alimentary; 32. Anginal-bubonic, abdominal; 33. Foothill-brook, soddy-alluvial-boggy; 34. Meadow-field, steppe.

Continuation of Table 23 [see next page]: 1-6. [Same as in legend above]; 7. Agricultural; 8. Domestic; 9. Food products; 10. Industrial; 11. Infection from processing agricultural products; 12. Infection from slaughtering animals and dressing the meat; 13. Common vole, house mouse; 14. House mouse, common vole; 15. House mouse; 16. Sheep and their ticks, hares; 17. Aspiration; 18. Thoracic (the term "thoracic form" proposed by German authors (by analogy with the abdominal form) eliminates the inconvenience of using such long terms as "tularemia with predominant involvement of the respiratory tract", used to date in the classification of this clinical form of tularemia (see Chapter VII)); 19. Alimentary; 20. Anginal-bubonic, abdominal; 21. Meadow-field, steppe; 22. Steppe, meadow-field, tugaic; 23. Steppe, meadow-field; 24. Contact; 25. Bubonic; 26. Cases occur outside the natural focus (importation); 27. Trench.

Infection in the laboratory.

The classification adopted in this Chapter has been created historically; the principles of its construction were laid down with the beginning of the study of tularemia. At the present time it is used by the majority of investigators and practical workers. Therefore, it would be inadvisable to change it radically. It would be better, in connection with the finding of new epidemiological rules and regulations, to make those changes which have been conditioned by the further study of tularemia epidemiology, which we have done.

Tularemia outbreaks are comparatively rarely untypical with respect to the conditions of infection; much more often they are characterized by inhomogeneity. For example, in the arthropod-borne outbreaks part of the infections can occur by water or by occupa-

Continuation of Table 23

Состояние статуса	Вид	Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Состояние статуса		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Вид		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Вид		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Вид		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Вид		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы
Вид		Объем материала	Плотность материала	Преобладающая литературная форма	Примечание к описанию литературной формы



tional routes; agricultural outbreaks can be associated with water and domestic infection, etc. In determining the nature of various outbreaks one needs to be oriented with respect to the predominant epidemiological type of disease, and if a subordinate type (or types) is of considerable importance we may speak of mixed outbreaks, for example, agricultural and domestic, occupational and arthropod-borne, etc. The various epidemiological types of tularemia are essentially different from one another with respect to seasonality and frequency of occurrence, age and occupational composition of the patients, etc. These problems will be discussed in greater detail in the analysis of the various types of disease (outbreaks).

#### Characterization of the Various Epidemiological Types of Disease

**Arthropod-Borne Type.** The cases belonging to this type occur from transmission of the tularemia pathogen to man by blood-sucking arthropods. These may be Diptera or ixodid ticks. In view of the essential differences in the conditions of infection between these two groups of vectors and, correspondingly, the difference in the necessary prophylactic measures the arthropod-borne type of disease can be divided into two variants:

Cases associated with the transmission of infection by blood-sucking Diptera (horseflies, mosquitoes). An outbreak of tularemia caused by infection through blood-sucking Diptera was first described by G. Ya. Sinay (1931, 1935) on the basis of observations which he made in conjunction with P. P. Popov in 1930 in Southeast Kazakhstan (the village of Ush-Tobe in what was formerly Taldy-Kurganskaya Oblast) as well as those made by A. Ya. Krol' (1933), who in the same year investigated a similar outbreak in what was formerly Barabinskiy Okrug (West Siberia). G. Ya. Sinay (1934) called this type of disease "spontaneous", but afterwards suggested the name "arthropod-borne" for it (N. G. Olsuf'yev, 1938, 1940).

Subsequently, outbreaks of this type were studied in detail by A. A. Miller and B. N. Stradomskiy (1935), P. V. Somov and co-authors (1940), S. P. Karpov and coauthors (1943, 1945), Yu. A. Isaakov and O. N. Sazonova (1946), L. M. Khatenever (1946), Ye. Bartoshevich (1948), V. G. Beletskiy (1948), Ye. I. Novikova (1951), M. I. Antsiferov and coauthors (1957), V. P. Borodin (1958), V. G. Pili-penko (1959) and others.

Apparently, the arthropod-borne type of tularemia is one of the oldest. Specifically, in the opinion of many investigators (Ye. I. Novikova, 1946; A. A. Maksimov, 1948; V. S. Sil'chenko, 1957 and others), a number of outbreaks observed on the territory of the Soviet

Union prior to 1930, including outbreaks near Astrakhan' in 1877 (M. I. Galanin, 1897) and 1926 (S. V. Suvorov and coauthors, 1928), on the Obi near the mouth of the Irtysh in 1921 (V. A. Anishchenko, 1922), etc., were arthropod-borne (for more details see Chapter I). Cases of arthropod origin are also known abroad, particularly in the United States and Sweden (see Chapter II). Outbreaks of the arthropod type were noted in various places of the Soviet Union: in the Ukraine, Belorussia, Kazakhstan, Western and Eastern Siberia (including Yakutiya and Krasnoyarskiy Kray), the northern and middle belts of the European portion of the USSR, in the deltas of the Volga, Don, and Terek rivers, etc.

Arthropod-borne outbreaks, caused by transmission of infection by blood-sucking Diptera, are associated in their occurrence and development with a diffuse epizootic among rodents, chiefly water rats. However, in various cases infection of insects can occur from hares or other rodents which can be bitten by them. The insects become infected from sucking the blood of sick animals, but there are indications to the effect that the infection of horseflies can also occur from the dead bodies of water rats as well as from water infected with the tularemia pathogen (N. G. O'suf'yev and D. A. Golov, 1940). Infection of people from arthropod-borne outbreaks is observed, as a rule, near water bodies, in river valleys, in meadows and saymishcha (a Siberian name for lowland marshes covered with reeds), where water rats live. Usually, infection occurs at the time of hay-making and gathering in the grain, in working in orchards, conducting improvement operations, processing peat, catching fish, etc.

The census of water rats and the development of tularemia epizootics among them have undergone considerable variations over a number of years (see Chapter V), as the result of which the conditions for the occurrence of arthropod-borne diseases are also changing over the years. A more frequent occurrence of cases of this type, sometimes for several years straight, has been observed in a number of places of Western Siberia and in the deltas of some southern rivers (for example, the Volga, Don), whereas in the central and northern oblasts of the European portion of the USSR arthropod-borne outbreaks were rarer. They were observed in the same place for no more than one season and then were absent for a long time. Infection of people with tularemia occurred through the bites of blood-sucking vectors infected with tularemia, by means of crushing the latter and rubbing their tissues into the skin (by scratches) or after entrance of the insects into the eye. Under natural conditions, in foci of tularemia mosquitoes spontaneously infected with tularemia bacteria (*Aedes*, *Culex*, *Anopheles*) as well as spontaneously infected horseflies (*Chrysops*,

*Chrysosoma*, *Tabanus*) have been found repeatedly, and in experiments on laboratory animals their part as vectors of the infection has been proved (see Chapter V). These observations as well as the correlation between the flight seasons of horseflies and mosquitoes and the seasonality of the arthropod-borne outbreaks which has been noted repeatedly (N. G. Olsuf'yev, 1940; V. P. Borodin, 1958 and others) show quite convincingly that in this type of disease these insects specifically are the vectors of tularemia. However, in some cases horseflies may be of predominant significance; in others, mosquitoes.

Arthropod-borne outbreaks usually begin in July or June, reach a maximum in August and stop in September or the beginning of October (Fig 40). Haytime and harvesting operations, which come about in the second half of July and August, contribute to the increase in the morbidity rate.

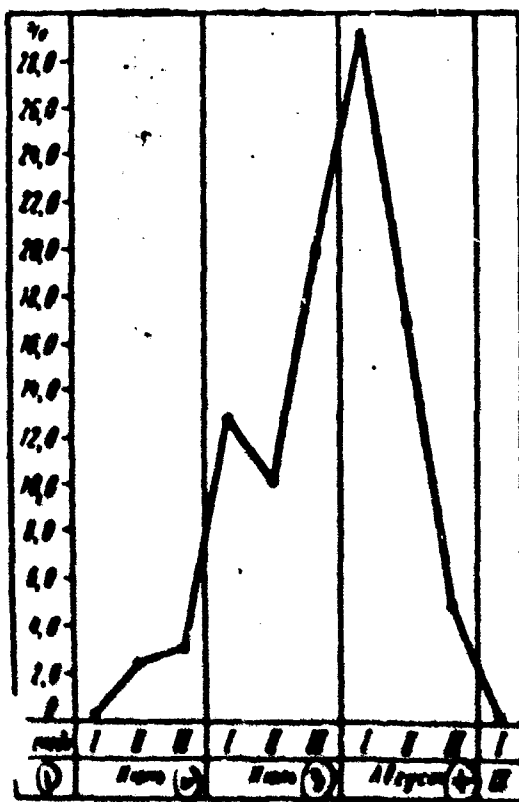


Fig 40. Movement of the Morbidity Rate by 10-Day Periods in an Arthropod-Borne Outbreak of Tularemia (after S. P. Karpov and coauthors, 1945). 1. 10-day periods; 2. June; 3. July; 4. August.

Outbreaks were usually local; they included the inhabitants of one or several inhabited places and amounted to a few score or hundreds of cases. However, a scattered distribution of cases is also known. For example, M. I. Antsiferov and coauthors (1957) noted that in one of the rayons of Krasnoyarskiy Kray in 1950, 202 cases of tularemia were recorded in 24 inhabited places. In West Siberia in various years large arthropod-borne outbreaks were noted which included a considerable number of inhabited places (S. P. Karpov and coauthors, 1945; Yu. A. Isakov and O. N. Sazonova, 1946; O. V. Ravdonikas, 1958).

In arthropod-borne outbreaks chiefly the adult population becomes sick which participates in various operations associated with being in river valleys, boggy meadows, etc.

The distribution of cases by sex and age usually quite accurately depicts the composition of the participants of these operations (S. P. Karpov, 1945; Ye. Bartoshevich, 1948 and others). However, if an inhabited place is located directly in a natural focus of the soddy-alluvial-boggy type cases can be observed beginning with early childhood and ending with advanced old age, that is, also among persons who do not leave the house (Ye. Bartoshevich, 1948). In these cases, evidently, infection occurs from infected mosquitoes or horseflies which fly into the inhabited place. V. G. Filipenko (1959) noted the role of winds in the passive transfer of infected blood-sucking Diptera (mosquitoes) far beyond the limits of the natural focus during a considerable arthropod-borne outbreak of tularemia in the delta of the Terek River in 1955.

Chiefly the exposed parts of the body are subjected to attack by blood-sucking Diptera. At the site of the bite, as a rule, a small ulcer forms which is localized in the area of the face, neck, upper or lower extremities. Depending on the site of the bite a regional lymphadenitis develops, usually in the cervical, parotid, submaxillary, axillary and inguinal areas. From time to time, also the ophthalmic-bubonic form is observed, which is explained apparently by transfer of the infection to the eyes by the hands after crushing the vectors or by the entrance of the insect into the eye.

Many authors have studied the localization of the primary inflammatory changes (small ulcers) and particularly buboes in various arthropod-borne outbreaks. It was determined that this localization to a considerable degree depends on the type of clothing worn by the local inhabitants. If the lower extremities cannot be reached by the insects (wearing trousers, boots), the small ulcer at the site of the portals of entry of the infection is usually located on the face, neck, hands and forearms with a corresponding localization of the regional

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lymphadenitis (bubo). If the lower extremities are exposed (wearing skirts, walking barefoot), the small ulcer is usually localized on the leg, and the bubo develops in the groin (V. G. Beletskiy, 1948). Cases are known where several small ulcers were found in the patients which were located in various places on the body, which is evidently connected with simultaneous multiple infection by the vectors.

The presence of a small ulcer on the skin is considered characteristic of an arthropod-borne infection. However, in different outbreaks the percentage of cases in which a primary lesion was found varied. Ye. Bartoshevich (1948) writes that he observed a primary lesion "in almost all patients"; V. G. Beletskiy (1948) found small ulcers in 92 percent of the patients; V. P. Borodin (1958), in 64 percent; G. Ya. Sinay (1935), in 60 percent. Ya. I. Belenkov (1948) observed an outbreak, judging from his description, which was undoubtedly of the arthropod-borne type, in which small ulcers at the sites of the portals of entry were found in only six percent of the patients. (If the examinations of the patients are not careful enough, particularly during the period of recovery, the primary lesion can easily be overlooked).

The author considered this outbreak a water outbreak and believed that infection occurred chiefly through the skin of the lower extremities during haymaking on the wet meadows. The editorial staff of the Journal of Microbiology, Epidemiology and Immunobiology directed attention to the incorrectness of this conclusion at the time Y. I. Belenkov's article was published.

In the case of arthropod-borne outbreaks sometimes patients with the anginal-bubonic or abdominal (intestinal) forms of tular-emia were noted. In these cases infection hardly occurred from the blood-sucking Diptera; rather, we may think of a water route of transmission (V. S. Sil'chenko, 1957), because during summer epizootics among water rats various bodies of water are infected with the tular-emia pathogen (F. G. Pilipenko, 1953 and others). No deaths have been noted from arthropod-borne infection in the USSR.

Cases associated with transmission of the infection by ixodid ticks. In contrast to the United States, where for a number of years quite a number of cases of tularemia infection from ticks was recorded (see Chapter II), in the USSR cases of this type are not often recorded. They were noted in the middle belt of the European portion of the RSFSR (Moskovskaya, Smolenskaya, Tul'skaya, Lipetskaya oblasts) and in the South (Stalingradskaya Oblast, Stavropol'skiy Kray, Armenia, what was formerly Taldy-Kurganskaya Oblast of the Kazakh-SSR). The occurrence of the majority of them was in natural foci of the meadow-field, forest and steppe (ravines) types.

At the present time, a total of 10 cases of tularemia in people have been described as the result of infection from ticks (A. K. Shanshiyev, 1944; N. G. Olsuf'yev and coauthors, 1949; V. N. Zil'fyan, 1951; Yu. A. Myasnikov, 1952; A. M. Sorina, 1955; V. P. Borodin and coauthors, 1956; M. A. Aykimbayev, 1959 and others). As vectors, mature *Dermacentor pictus*, *D. marginatus*, *Ixodes ricinus* and *Rhipicephalus rossicus* ticks were noted. The infection occurred during the course of attachment of the ticks to the human body (on questioning the patient this fact was usually established comparatively easily), and the disease usually occurred in the form of the ulcerative-bubonic form. Localization of the primary lesion (small ulcer) differed in accordance with the site of attachment of the ticks, while the latter to a certain degree depended on species characteristics of these parasites. In some cases it was the inguinal area or lower extremities; in other cases, the axillary region, etc. In accordance with this, buboes were observed in the groin, axilla, etc. The conditions for the infection were more or less of the same type -- work (taking cattle out to graze or care of them, wood-cutting, etc.) or being accidentally in habitats of ticks. In one case the infected ticks were brought into the house with dry twigs (Yu. A. Myasnikov, 1952). Cases were noted from May through August, that is, in the season of tick activity.

N. G. Olsuf'yev (personal report) observed a case of tularemia in an adolescent who was infected with dermacentor ticks while taking them off a cow and then crushing them with a stone in the South of Moskovskaya Oblast (Mikhnevskiy Rayon) in May 1948. The infection was transferred to the eyes by the contaminated fingers, and the patient had a typical ophthalmic-bubonic form of tularemia. Such cases have been observed and described repeatedly in the United States.

In places of occurrence of cases of tularemia from tick infection cultures of the pathogen of this disease were repeatedly isolated from the ticks (V. P. Borodin and coauthors, 1956; V. N. Zil'fyan, 1951 and others), which confirms the role of ticks as vectors. Ixodid ticks are infected from rodents sick with tularemia usually on this such as gray voles, hares, hamsters, and partly from water rats. By preserving the tularemia bacteria in their bodies for a long time the ticks can be responsible for the infection of people even if, at the given moment, there is no tularemia epizootic among the rodents. The number of infected ticks in the focus usually does not exceed one percent, but in various areas it can reach five and six percent (N. G. Olsuf'yev and Ye. N. Tolstukhina, 1949; V. P. Borodin and coauthors, 1958). The ability of the ticks of the species listed to transmit or preserve tularemia infection has been shown experimentally in laboratory animals (see Chapter V).

Apparently, in the USSR cases of tularemia in people as the result of tick infection occurred more often than they were recorded, because the diagnosis of sporadic cases of tularemia was made inadequately in the past (Yu. A. Myasnikov, 1955).

Cases of allergy which can occur in those people vaccinated against tularemia when naturally infected ticks attach themselves to them deserve attention. Two such cases, associated with the bites of *Rhipicephalus rossicus* ticks, have been described by V. P. Borodin and coauthors (1958). In both patients a two-three-day temperature elevation to 37.2-37.5° was observed, and at the site of the bite redness and edema of the skin appeared over an area five-six centimeters in diameter, and there was also pain or itching. In one case pain and enlargement of the regional lymph node were also noted. A culture of the tularemia pathogen was isolated by a biological test from the ticks removed. These data indicate the fact that persons inoculated should take precautions against tick bites during work in foci of tularemia and should observe measures of personal prophylaxis.

**Occupational Type.** Cases belonging to this type occurred in the water rat, muskrat and other animal industries with the aim of preparing their pelts for fur. Chiefly hunters became sick and in some cases members of their families who assisted in removing and processing the pelts from the animals caught became sick also. Cases of tularemia associated with hunting and the consumption of hares as food are distinguished as a separate epidemiological type by us as well as other authors, because hunting hares is usually not of the nature of an occupation, and infection usually occurs by the alimentary route and includes separate families. Existing data on the epidemiology of occupational diseases are being analyzed in accordance with the species of animals involved in the occupation.

**Infection in the Water Rat Industry.** Outbreaks of tularemia, associated with the water rat industry, were noted for the first time in the USSR in 1927-1928 almost simultaneously in a number of places (Ryazanskaya, Orenburgskaya, Tyumenskaya, and other oblasts). The number of cases among people was considerable, for example, an outbreak in Ryazanskaya Oblast amounted to 800 cases (see Chapter I). The organization of a large-scale water rat industry contributed to the occurrence of large tularemia outbreaks; this was nonexistent prior to 1927. A high census of the water rat and the relative ease with which it is caught, particularly during a flood period, attracted many people living in villages near their habitats to this occupation. The number of pelts obtained yearly amounted to several million. In subsequent years the occupational type of disease was noted repeatedly in many places of the territory of the USSR, where water

rats are numerous. Outbreaks of tularemia associated with the water rat industry have been described in detail by A. A. Vol'ferts (1928), D. A. Golov and coauthors (1928), G. I. Zarkhi (1929, 1930), A. F. Komarova (1945) and others.

The water rat industry occurs chiefly during the period of the spring river overflow. At this time, the animals have to leave their permanent holes and accumulate in large numbers on non-inundated highlands, the tops of bushes, trees, and are easily caught. Therefore, occupational outbreaks were usually observed in the spring, during the overflow period, and in rivers with a late overflow they were noted at the beginning of the summer. In West Siberia, and, in places of the European part of the USSR, an autumn water rat industry exists, and at this time cases of infection of people with tularemia have also been noted from the rats caught (A. F. Komarova, 1945). Infection of people with tularemia usually occurred as the result of contact with sick water rats, their carcasses and pelts. Various cases of infection through the bite of a rat have been noted. The duration of outbreaks of this kind depended on the duration of the hunting season. At the end of this hunting season the cases stopped. The number of cases was determined by the intensity of epizootics among water rats, the census of these latter in a given locality and the number of persons occupied in the industry. Not uncommonly, when the majority of inhabitants of an inhabited place participated in catching the water rats, skinning them and further processing of the pelts cases of tularemia assumed a mass nature. Thereby, not only the adult population but even children in contact with the contaminated objects became sick. Adolescents, who usually participate in the water rat industry, are subject to tularemia infection on a par with adults.

In connection with the fact that tularemia usually occurs by means of contact with sick water rats and their pelts, carcasses or contaminated objects, characteristic of this type of outbreak is the ulcerative-bubonic or purely bubonic form. The portals of entry of the infection are frequently slight scratches on the skin of the hands; therefore, most often axillary buboes are observed. During removal of the pelts or contact with the carcasses of the rodents the tularemia microbe can be transferred to the conjunctiva of the eye by contaminated hands, to the oral cavity and to other parts of the body. Therefore, ophthalmic, anginal and other clinical forms of tularemia can occur.

Some believe that in the case of occupational outbreaks the infection can occur through rodent ectoparasites (gamasid ticks, fleas, lice) from bites or from crushing these insects on the body; however, the probability of such infection is extremely slight. We have not



found any accurately established facts of infection of people under circumstances of this kind in the literature. However, during the water rat hunt and industry, cases of infection of people were noted through infected water or blood-sucking Diptera.

Inadequately dried pelts of water rats can present a danger of infection when they are given to storage points (P. V. Somov and co-authors, 1939; A. F. Komarova, 1945).

At the present time, as the result of compulsory vaccination of all persons participating in the industry and their family members as well as the taking of other measures, this type of case is practically not observed.

**Infection in the Muskrat Industry.** Cases of tularemia in people from hunting muskrats have been noted repeatedly in the United States and Canada, but only in the form of sporadic cases (see Chapter II). In the USSR acclimatization of the muskrat began in 1928, and at the present time this valuable fur-bearing animal has become a large-scale hunting object in many places of the country. Special muskrat-hunting economies are engaged in observation of the multiplication of the muskrat and catching it.

The first case of infection of man with tularemia from a muskrat was noted in 1937 in West Siberia (B. V. Voskresenskiy, 1943). Several cases of transmission of disease from muskrats were then observed in Novosibirskaya Oblast in 1939 (A. F. Komarova, 1945). Subsequently, cases of infection of people from this type of rodent were recorded in Buryat-Mongol'skaya ASSR in the Selenge delta and the lowlands of the Upper Angara (T. G. Linnik, 1957; M. I. Antsiferov and coauthors, 1957), in KazakhSSR in the Syr-Dar' delta (L. S. Kamennova and V. M. Smirin, 1959) and other places. Cases occurred in the autumn, winter or spring during the period of muskrat hunting and usually had the nature of small outbreaks which numbered half-score or a score of cases. With the end of the hunting season there were no new cases. The outbreaks were preceded by a tularemia epizootic among muskrats, which was determined bacteriologically.

The transmission of tularemia from the muskrat to man occurs chiefly under the same conditions as in the water rat industry. The mechanism of infection is the same, that is, mainly contact, but in various cases the infection could occur from the consumption of muskrat meat as food or the drinking of unpurified water from bodies of water where the muskrat lived (T. G. Linnik). In the clinical picture the bubonic form predominated, but from time to time the abdominal form was observed. The relatively local character of the tularemia epizootics among muskrats (see Chapter V) limits the epidemiological significance of this species of rodent. In addition, hunting

the muskrat is usually accomplished in an organized manner; a comparatively small number of hunters participates in it, which limits the possibility of infection of the population and facilitates the accomplishment of a combination of prophylactic measures. The latter are the same for muskrat and water rat industries (see Chapter XI). In places where both types of industries are well developed, these measures are conducted in a standardized manner, considering the fact that the same persons are usually engaged in the muskrat and water rat industries.

**Infection from Hunting Other Species of Animals.** On the territory of the USSR the pelt industry is well developed with respect to sousliks, squirrels, marmots, hamsters, moles, ermines, polecats and other mammals. However, these species of animals are relatively insensitive to tularemia (they belong to groups II and III) in their majority; they become sick with a relatively sparse seeding of the organs and tissues with tularemia bacteria and are involved in the epizootic only by accident (see Chapter V), which limits their epidemiological significance. V. A. Bernikov and coauthors (1935) described a case of tularemia occurring as the result of a souslik bite (in the village of Ust'-Kurduyn in Saratovskaya Oblast). A. A. Selezneva (1949) observed cases of tularemia in 1941 in one of the rayons of Gorno-Altayskaya autonomous oblast associated with hunting and the consumption of the meat of long-tailed sousliks, *Citellus undulatus*, as food with simultaneous infection from infected water sources through drinking water. On epizootological investigation cultures of the tularemia microbe were isolated (by biological test) from the long-tailed souslik and water rat. One has to reckon with the possibility of mechanical transfer of infection through the bites of carnivores (group III), the oral cavities of which are infected from eating rodents sick with tularemia. Such cases have been noted repeatedly abroad among hunters and have been confirmed experimentally in polecats and guinea pigs (N. G. Olsuf'yev and T. N. Dunayeva, 1951).

Hunting animals highly sensitive to tularemia (group I), for example, hamsters and moles, can be of indubitable epidemiological importance. In 1953, V. P. Bozhenko and coauthors (1955) observed cases of tularemia in people in one of the steppe rayons (Rostovskaya Oblast) associated with hunting the Precaucasian hamster (*Mesocricetus raddei*). Cases of tularemia from hunting the common hamster (*Cricetus cricetus*) have been observed in the Ukraine by S. N. Ruchkovskiy and coauthors (1935), and recently they have been noted in Roumania (see Chapter II). Yu. A. Myasnikov (1955) reported two cases of tularemia in people engaged in mole hunting (*Talpa europaea*) in Tul'skaya Oblast. The cases listed indicate the need for taking prophylactic measures in hunting these species of animals.

**Hunting-Food Type.** In this type of case we include those associated with hunting hares, removing their pelts, as well as dressing and eating their carcasses. In the majority of foreign countries hares are the main source of tularemia in people (see Chapter II). In the USSR cases of tularemia in people from hares were first found by I. F. Berezin (1931, 1934), but outbreaks which he described should be considered industrial (see below). Sporadic cases or individual outbreaks of tularemia occurring as the result of hunting hares and consumption of their meat as food were noted in many places of the Soviet Union, including the Ukraine, Belorussia, Moldavia, the middle belt of the European part of the USSR (Moskovskaya, Tul'skaya, Smolenskaya and other oblasts), in the North Caucasus, in Western and Eastern Siberia, Kazakhstan, etc. They were described by S. N. Ruchkovskiy and coauthors (1935), V. N. Ter-Vartanov and coauthors (1943), I. N. Mayskiy (1944), G. P. Slavin (1946), A. A. Selezneva (1950), I. R. Drobinskiy (1951), M. F. Shmuter (1958), Yu. A. Myasnikov and M. I. Tsareva (1959) and others. In our subsequent presentation we shall use chiefly the data of Yu. A. Myasnikov and M. I. Tsareva, who generalized on materials on the hunting-food outbreaks in the RSFSR.

In the central and western oblasts of the RSFSR, in the North Caucasus and in the Ukraine (M. F. Shmuter, 1958) the main source of infection is the gray hare (*Lepus europaeus*); in the northeast of the European part of the RSFSR, in West Siberia and in Yakutskaya ASSR the white hare plays an epidemiological role (*Lepus timidus*). In Southern Kazakhstan cases of infection of people have been noted from the Central Asiatic hare (*Lepus tolai*) (N. F. Kalacheva and coauthors, 1957). Cases from hares in people have been observed on the territory of the RSFSR every year (the data for 1946-1955). More than one-third of the annual morbidity from gray hares and one-half of the morbidity from white hares occurred in the spring, although at this time hunting hares is forbidden and catching them is of a happenstance nature (poaching, catching sick hares, etc.). In the spring a tularemia epizootic among hares is apparently at its peak. It is associated at that time with the attacks of infected mature ixodid ticks on the animals. In the summer the number of cases from hares occurring in people is markedly reduced and is increased somewhat in the autumn, at the beginning of the hunting season. The third peak in the morbidity, but only in places where the gray hare is common, occurs in the winter months. This rise basically is observed in the years in which there is a high census of small mouse-like rodents and tularemia epizootics among them. The infection of hares in the winter occurs usually during feedings at hay and straw stacks, in which there are voles or house

mice sick with tularemia which by their excretions infect the straw and the hay. In places where white hares are common, winter cases of infection of people from hares are not observed, which is explained by the ecological characteristics of this species of hare, which lives in the woods and, as a rule, has no contact with the common vole. In addition, in the northern rayons of the European part of the USSR in Siberia, where the white hare lives, winter epizootics among the common and other voles are not massive. Depending on the latitude of the locality and in accordance with the time of development of tularemia epizootics among small mouse-like rodents and transfer of the infection to the gray hares the cases of the hunting-food type can be shifted in time. For example, in Kaliningradskaya Oblast, in the south of the Ukraine and in the North Caucasus infection from hares was observed chiefly in November-December; in the north of the Ukraine and in the middle belt of the RSFSR the maximum number of cases occurred in December-January-February. In various years the proportion of cases from hares in the total tularemia morbidity rate amounted to one-four percent in the middle belt of the European part of the RSFSR; two percent, in Khar'kovskaya Oblast (in Khar'kov 30-40 percent according to M. F. Shmuter); in Kaliningradskaya Oblast it reached 64 percent.

Study of cases of infection of people from hares shows that the epidemic hazard is constituted by animals sick with tularemia which can be caught by hand or killed with a stick. Characteristic of the "hare" outbreak is familial disease, which is found chiefly in those families in which there are hare hunters. According to the data of Yu. A. Myasnikov and M. I. Tsareva, only in 15 percent, that is, in less than one-sixth of all cases, did the hunters alone become sick. The number of people infected from a single hare can differ and sometimes reaches 10. All age groups are equally infected, whereby cases of disease in two-three-year-old children are observed. The infection occurs most often from skinning the animal, eviscerating and dressing the hare carcass. Thereby, the pathogen usually penetrates through the skin of the hands, on which there may be abrasions sometimes unnoticeable by the naked eye. However, in part of the cases the infection occurs by the alimentary route, with the consumption of inadequately cooked hare meat as food. Cases of infection of people from salted hare carcasses have been noted (I. F. Berezin, 1934).

In the type of case being analyzed the ulcerative-bubonic or purely bubonic form (50-60 percent) predominates with an enlargement of antecubital or axillary lymph nodes and the localization of the small ulcers (in the ulcerative-bubonic form) on the skin of the hands (usually on the fingers). Abdominal (21-29 percent) and anginal-bubonic (11 percent) forms are also quite frequent, and from time to time

ophthalmic-bubonic or thoracic forms of tularemia were observed.

Usually a severe course of the disease is noted, and in the case of alimentary infection it can be particularly severe, sometimes with a fatal outcome (T. A. Rubina, 1957; M. F. Shmuter, 1958; see also Chapter VII). The latter should be connected with the massive dose of infection, because in hares, as in other animals of group I, tularemia occurs with intense seeding (+++-+++) of organs and tissues with bacteria.

In the literature cases of tularemia among people have been described occurring apparently as the result of consumption of the meat of other types of game as food, particularly, bear, the Central Asian gazelle and wild boar (I. R. Drobinskiy and V. K. Klimukhin, 1948). However, these cases need more exact proof, because ungulates and carnivores, to which these species of animals belong, are very resistant to tularemia (see Chapter V).

**Water Type.** The role of water as a factor in the transmission of tularemia from sick rodents to people was first pointed out by Soviet investigators B. N. Starodomskiy, I. S. Tinker, P. V. Somov, A. A. Vol'ferts, Ye. I. Novikova, S. P. Karpov and others, which was later confirmed in the United States, Turkey and other countries. The water route of transmission of the tularemia microbe is associated with considerable stability of the latter in water, particularly at low temperature (see Chapter III), as well as with the fact that species of rodents which are sources of infection live along the shores of open water bodies, or it is associated with the chance entrance of rodents sick with tularemia into sources of water supply (wells, etc.).

Data which have been accumulated at present concerning the water type of tularemia make it possible to distinguish two epidemiologically different variants: a) infection through the water of brooks and other open sources of water and b) infection through well water.

**Infection through the Water of Brooks and Other Open Sources of Water.** An outbreak of tularemia caused by infection from the water of a brook was first observed in 1935 in West Siberia (formerly Tynginskii, now Yashkinskiy Rayon of Kemerovskaya Oblast) by S. P. Karpov and N. I. Antonov (1936). The cases occurred at the beginning of July during haymaking from the consumption of the water for drinking purposes (or washing). Cultures of the tularemia pathogen were isolated from the water by guinea pig passage. Subsequent cases of this kind were noted repeatedly, whereby it was determined that they are most characteristic of the natural focus of the foot-and-brook (water according to S. P. Karpov) type, where the proportion of them amounts to 98 percent of the entire incidence of tularemia (I. I. Kuzina, 1959). Most often, these outbreaks were observed in

Kemerovskaya Oblast, Altayskiy Kray, Vostochno-Kazakhstanskaya Oblast and in the Maykop region of Krasnodarskiy Kray.

Infection of brooks occurs chiefly from water rats sick with tularemia (or animals which have died), whereby the pathogen can be found in the water of the brooks sometimes for several months, to which water-dwelling animals contributes (see Chapter V), in the opinion of S. P. Karpov and A. A. Selezneva. The presence of the tularemia pathogen in the water of rivulets and other open bodies of water has been proved repeatedly by various authors by means of isolation of corresponding cultures from a biological test.

Cases among people usually occurred in the summer, with the maximum in July or August, whereby the peak is associated with haymaking and other field work, during which the population makes extensive use of water from brooks for drinking. Cases of infection have also been noted through the skin, for example, in washing, bathing, walking barefoot through the brook, doing the laundry, etc., but the importance of this route of transmission of the infection in water outbreaks is usually small by comparison with alimentary outbreaks. In foci of the foothill-brook type water outbreaks were noted not only in the summer but also in the winter (T. D. Romashova, 1959). In view of the relative stability of foci of the foothill-brook type they represent a constant epidemic hazard, and the prophylaxis of disease in them should be conducted particularly persistently.

Such infections among people have been observed quite often also in natural foci of the soddy-alluvial-boggy type, for example, in Omskaya Oblast during the period from 1946 through 1955 they amounted to 22 percent of the entire incidence of tularemia (O. V. Raydonikar, 1958). In Voronezhskaya Oblast, in the natural foci of this type water infection has also been noted. These cases have been observed in the summer and at the beginning of autumn, frequently simultaneously with arthropod-borne outbreaks, and the infection occurred either by the alimentary route from drinking water from open bodies of water (ponds, natural pools, etc.) or during bathing in ponds, lakes, rivers, in walking through these bodies of water barefoot, washing, doing the laundry, etc. (V. S. Sil'chenko, 1957). In Moskovskaya Oblast outbreaks have been noted repeatedly in connection with infection from the water of springs or brooks, whereby it was characteristic that they were observed only in the winter. These outbreaks occurred in a relatively inactive manner, and the number of patients was small (M. I. Tsareva, 1959). Winter outbreaks of tularemia have also been described occurring from the consumption of water from a commercial water supply system. In these cases the water came from open bodies of water (ponds, etc.) and was not chlorinated (M. I. Tsar-

eva, 1945, 1959; S. P. Karpov, 1950). During these outbreaks the tularemia pathogens were isolated from samples of water taken not only from the water supply system but also from a water body from which the water came.

Cases have been noted of tularemia as the result of the attachment of leeches (S. N. Ruchkovskiy and coauthors, 1935; V. S. Sil'chenko, 1950) during a walk in the water through marshy places. In these cases the role of infected water has not been ruled out completely, because in the experiment it was not possible to transmit tularemia through leeches (V. P. Romanova, 1949). It is possible that leeches, by impairing the integrity of the skin at the time of their attachment, contribute to the penetration of tularemia bacteria into the wound from the water. In the United States, for example, a case of infection of man with tularemia through a puncture by the fin of a fish caught in the river has been described (Miller, 1939).

The water of small open bodies of water as well as of dam sites from which water is used in the water supply system, can be infected by small mouse-like rodents also during the period of their high census and active epizootics, which we repeatedly observed in Stavropol'skiy Kray in 1940. The tularemia pathogen at that time was isolated in Nagutskiy Rayon from a pond the water of which was used by the population. Cultures of the tularemia microbe were also isolated from a single water supply line and a small brook.

Winter water-borne outbreaks occur somewhat differently from summer ones. Summer outbreaks are of an explosion-like nature; winter outbreaks occur more slowly, without great peaks. Thus, an outbreak described by S. P. Karpov, dragged on from December to the end of February: 10.2 percent of the patients were recorded in December; 60.3 percent, in January; 29.5 percent, in February. V. S. Sil'chenko (1957) believes that the duration and character of the course of water-borne outbreaks depend on the rapidity with which the focus of infection is detected and with which antiepidemic measures are taken rather than on the time of the year and the type of water source.

In the case of water outbreaks described above there was a predominance of the anginal-bubonic form of tularemia. S. P. Karpov (1950) reports that in the outbreak which he studied in 1935 the anginal-bubonic form was present in 67.5 percent of the cases; in 16.3 percent, the purely bubonic form; in 4.6 percent, the ophthalmic-bubonic form and in 11.6 percent, the intestinal form of tularemia. A. A. Selezneva (1948), who studied the morbidity rate associated with water consumption from a single brook during the period from 1939 through 1946, determined the fact that in 81.3 percent the anginal-bubonic form occurred; in 11.4 percent, the ophthalmic-bubonic;

in 2.5 percent, purely bubonic (with localization of the buboes in the inguinal and axillary regions); in 1.8 percent, the intestinal form, and in three percent, the pulmonary form of tularemia.

In the case of water-borne infection the ages of the patients may be most different; specifically, cases have been observed where children beginning with a year of age have been infected (M. I. Tsareva, 1959).

N. G. Olsuf'yev and coauthors (1959) observed two cases of allergic disease in those vaccinated against tularemia lasting one-two days after drinking water from a brook which proved to be infected with the tularemia microbe (several cultures of this pathogen were isolated from the water).

**Infection through Well Water.** A well outbreak of tularemia was first described by P. V. Somov (1937); then, this type of disease was studied by us (1944), N. A. Popov (1947), V. S. Sil'chenko (1957), M. I. Tsareva (1959) and others. Usually, the well outbreaks occurred during a period of mass appearance of small mouse-like rodents (common voles and house mice) and the development of an acute tularemic epizootic among them. Frequently, agricultural and domestic types of outbreaks were noted simultaneously. Infection of people occurred from the consumption of water from the wells for drinking or from washing in this water, after rodents sick with tularemia had by accident entered the well. In the South well-type outbreaks were observed in the autumn and in the winter; in the middle belt of the European part of the RSFSR, in the winter or early spring. The time of their occurrence to a certain degree depended on the time the peak census of rodents was reached, as well as on the intensity of migration of the latter. Some authors have explained the entrance of sick animals into the wells by their "need to drink" but this is incorrect, because in the winter the moisture on the surface of the soil and in the fodder is entirely adequate for rodents. During their migrations rodents mechanically come into the wells (if there is a fault in them), just as into any other depressions in the ground (ditches, trenches, etc.) encountered along their line of movement. The following epidemiological indices are characteristic of infection from well water (I. N. Mayskiy, 1945): 1) the cases are limited to the group of persons who use an infected source of water supply regardless of age, sex or occupation; 2) the cases occur in a very short time and after the infected source has stopped being used (or in the case of disinfection of it) the outbreak terminates rapidly; 3) the predominant clinical form of disease in this kind of outbreak is the anginal-bubonic form of tularemia.

An outbreak occurring in February in one of the sovkhoses of Rostovskaya Oblast against the background of a tularemia epizootic



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in small mouse-like rodents (P. V. Somov, 1937) can serve as an illustration of this type of case. In a period of 22 days 242 persons became sick, which amounted to about 40 percent of the inhabitants of the village who used water from the well. Infection of the water was proved by isolation of the tularemia microbe from it twice (by the biological test). The outbreak was of an explosive nature (Fig 41) and included all age groups; particularly numerous were patients of school and pre-school (from one to seven years) age. The anginal-bubonic form of tularemia was predominant.

A large number of cases associated with the consumption of infected water from wells was observed in 1940 during a tularemia outbreak in Stavropol'skiy Kray. Thereby, we found many wells infected with the tularemia pathogen. In their majority it was possible to show the bodies of rodents which had fallen into the wells because of defective linings or the absence of covers.

We and N. N. Litvinov, L. M. Khatenover and others have noted the role of well water in the spread of tularemia in Stalingradskaya Oblast in the autumn of 1942 and in the winter of 1942/43 during the period of the defense of Stalingrad.

On the territory of this Oblast in the autumn of 1942 the mass appearance of mouse-like rodents occurred. The development of military operations on this territory considerably complicated the accomplishment of agricultural operations and taking agrotechnical measures, which contributed to rodent multiplication. The mouse census reached high figures. In living quarters 70-80 rodents a night were caught per 100 traps; in the field, 20 animals a night. Against the background of the high rodent census a tularemia epizootic occurred and after this there was an outbreak among people. Although the lines of movement of the infection in this outbreak were different, the water route of infection, particularly through well water, was of great importance. The predominant clinical form was the intestinal form. According to the classification existing at that time it was called the "generalized" form. The fact that in 61 percent of the cases intestinal disorders were noted (constipation, diarrhea, etc.) is evidence of the fact that this was actually an intestinal form of tularemia. Thereby, a large number of cases of angina were observed, as the result of which many local physicians who were not well acquainted at that time with the clinical aspects of tularemia made the diagnosis of influenza and angina. The extensive spread of angina at that time also was evidence of the fact that the water route of spread of the infection in this outbreak played a great part. Disinfection of the water of wells always led to a rapid reduction in the incidence of tularemia.

K. F. Akinfiyev (1955) also emphasizes the role of the

water factor in the epidemiology of tularemia during the years of the Second World War.

He notes that tularemia infection under conditions of combat operations occurred not only through well or brook water into which the dead bodies and excretions of the rodents fell but also through snow water as the result of contamination of the snow cover with rodent excretions. Snow water not uncommonly was used for drinking and domestic needs under conditions of wartime. From the materials of questionnaires of the patients made by A. I. Volkov, Ya. I. Belenkiy and B. Ye. Nesgovorov it was made clear that 41 percent of the patients used water from the river for washing; 43 percent, snow water; and 16 percent, well water. For the purpose of drinking 43.7 percent used river water; 38.3 percent, snow water; 19 percent, well water.

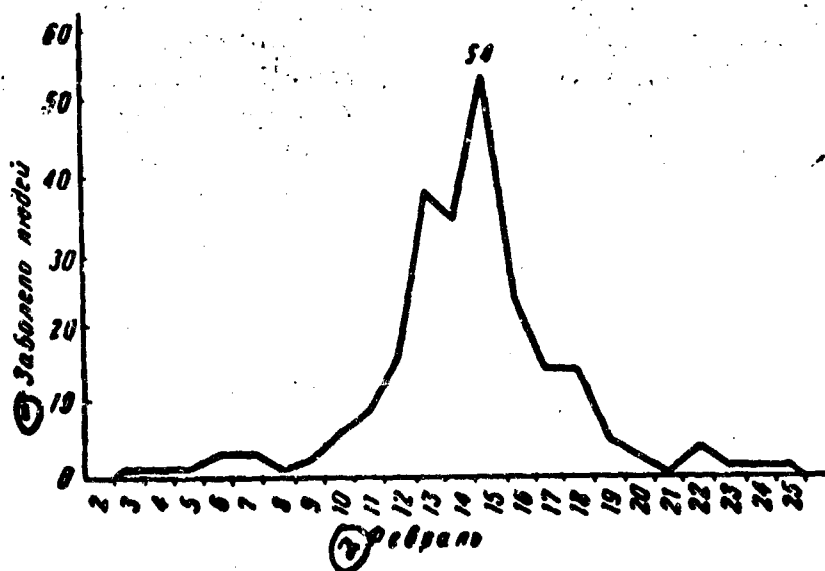


Fig 41. Movement of the Morbidity by Days in the Case of the Water (Well) Tularemia Outbreak (After P. V. Somov, 1937). 1. No. of people who became sick; 2. February.

In various cases, when the wells were located in the area of a natural focus of the soddy-alluvial-boggy type the source of infection of the water was constituted by water rats (S. P. Karpov, 1950;

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V. S. Sil'chenko, 1957). V. S. Sil'chenko (1957), after generalizing on data on water outbreaks of tularemia in Voronezhskaya Oblast, noted that in water-borne infection of people by the alimentary route the anginal-bubonic form was noted in 62-67 percent; the abdominal form, in 33-38 percent of the cases. Cases of angina were unilateral (76-82 percent) and, less often, bilateral (18-24 percent). In a number of cases the author observed the mixed form of tularemia -- anginal-bubonic and abdominal, which finds its confirmation in experimental studies on guinea pigs (R. A. Savel'yeva, 1958). Lesions of the mucous membrane under the tongue and in the lymph nodes of the floor of the mouth were found by V. S. Sil'chenko in seven-12 percent of the patients (simultaneously with the other forms) of those who cleaned their teeth and rinsed their mouths with unboiled water from infected sources. At the present time, in connection with the marked limitation of mass multiplication of small mouse-like rodents under the influence of the increased agrotechnical measures as well as by virtue of the improvement in the sanitary status of wells and mass vaccination of the population the possibility of occurrence of well-borne outbreaks of tularemia has been reduced sharply. However, prophylaxis should be carried out in full measure (see Chapter XI).

**Agricultural Type.** Tularemia outbreaks of agricultural type usually occurred at the same time as a period of mass multiplication of small mouse-like rodents, chiefly common voles and house mice, and the development of a tularemia epizootic among them. In some places in the Soviet Union where these species of rodents are few or entirely absent, water rats (see below) were noted in the occurrence of agricultural cases as sources of the infection.

An agricultural outbreak of tularemia occurring simultaneously with a domestic outbreak was observed for the first time in the winter of 1933-1934 in Rostovskaya Oblast (Salskiye Step'i) as well as in the southern rayons of Stalingradskaya Oblast and was described by V. A. Berdnikov (1934), A. A. Miller (1937), P. V. Somov (1937) and others. It began in October, reached the maximum in December, and ended in February-March, including the population of a considerable territory of these oblasts. Initially, the outbreak was considered an epidemic of influenza, and only in January, when it began to decline, was the correct diagnosis of tularemia made. Basically, house mice were sources of the infection. A. A. Maksimov (1948) believes that cases of the agricultural type and associated domestic infections occurred in the Soviet Union previously also, including in the period before the revolution, but remained undiagnosed. Abroad, cases of this kind have been noted in Roumania and quite recently in the United States (see Chapter II).

In the pre-War years as well as during the years of the Second World War and during the first years after the War agricultural outbreaks associated in their origin with small mouse-like rodents were observed in many places of the European part of the USSR; to the north, in Leningradskaya, Vologodskaya and Kostromskaya oblasts; to the south, including Moldavia, the Ukraine and the North Caucasus. Territorially, they coincided with the natural foci of the meadow-field and steppe types. During the years of the Second World War, when the harvesting of cereals on the fields as well as the threshing of ricks was delayed in a number of cases because of military operations or a shortage of help and agrotechnical measures were reduced, in places particularly severe rises in the census of small mouse-like rodents were observed associated with tularemia epizootics. Agricultural and domestic outbreaks occurring against this background were intense, particularly in the middle belt of the European part of the USSR (Tambovskaya, Ryazanskaya, Moskovskaya, Tul'skaya, Orlovskaya, Voronezhskaya and other oblasts).

Agricultural outbreaks of the pre-War and War years were studied by B. V. Voskresenskiy (1940), G. P. Slavin (1946), N. V. Nekipelov (1946), L. V. Gromashevskiy (1947) and others. The observations showed that the occurrence of agricultural outbreaks was associated with a concentration of rodents, chiefly the common vole (and in the South the house mouse as well) in the corn-stacks, hay ricks and straw-stacks, root cellars, granaries, etc. Persons participating in the stacking and carting of hay and straw, in winnowing and sorting the grain, as well as in other agricultural operations, were subject to infection.

In the type of disease being analyzed a special place was occupied by the so-called "threshing" outbreaks of tularemia which occurred in cases of delayed (winter or early spring) threshing of the ricks. A large-scale delay of threshing of cereal crops after harvesting the fields and stacking represented a special epidemic hazard, because in the stacks, when they stood a long time, additional favorable conditions were created for rodent multiplication and for the development of tularemia epizootics among them. During the threshing of such stacks a large number of living and dead rodents, including those which had died of tularemia (see Chapter V) were found repeatedly in them.

While settling in the stacks, corn-stacks and ricks, rodents sick with tularemia contaminate the straw, grain and hay with their excretions (urine, stool), and these substrates can remain infected for a long time because of the well-known resistance of the tularemia microbe to the environment, particularly in the presence of a low

environmental temperature. In the study of one winter epizootic of tularemia among voles in Tul'skaya Oblast the preservation of the tularemia pathogen in the straw stacks was followed for several winter months, and natural disinfection of the straw occurred only at the end of spring with the advent of warm weather. The presence of the pathogen in the straw was determined by means of a biological examination of smears from it and subsequent isolation of a culture (L. A. Poman-skaya, 1957).

In the case of agricultural outbreaks infection of people usually occurred from inhaling dust raised into the air from the infected straw, grain and other substrates when they were processed by machine or by hand, cleaned, transported, etc. However, in various cases during the accomplishment of agricultural operations the infection occurred by the alimentary (eating pea seeds, sunflower seeds, hemp, and taking food with the unwashed hands) or by the contact route (contact with the bodies of rodents, bite of a rodent, carriage of the infection with dirty hands or in dust to the mucosa of the eye, puncture of the skin with the straw, etc.). Cases of the agricultural type occurred from November through June with a maximum in January-March (in the south, the peak was in December). As a rare exception, the occurrence of cases associated with threshing of cereals was once observed in the middle of September (M. M. Gerasimova, 1954, Kalininskaya Oblast).

Cases of the type under analysis were usually noted among persons engaged in doing general work, whereby they could occur even in the absence of late threshing. Infections during threshing were usually characterized by the occurrence of mass outbreaks in a short time. The time of appearance of the "threshing" outbreaks depended on the beginning of the threshing of the stacks in which the rodents were involved in the tularemia epizootic. In the absence of delayed threshing cases of outbreaks of the agricultural type were less numerous and usually occurred at different times. By and large, persons at an age capable of doing work became sick, particularly kolkhoz members (especially the field- or animal-husbandry brigades, MTS [Machine and Tractor Station] workers, workers of the sovkhoses and subsidiary economies). However, other population groups became sick also if they participated in the agricultural operations.

The census of small mouse-like rodents and the development of tularemia epizootics among them underwent considerable fluctuation over a number of years, in connection with which the conditions for the occurrence of cases of the agricultural type also changed. In Tul'skaya Oblast, for example, the increase in the census of common voles and the development of tularemia epizootics among them were

observed usually once every three years; in the south, these increases were even less often. In accordance with this, cases of the agricultural type showed the same rhythm in their occurrence, being frequently completely absent in the intermediate years.

In the type of disease being analyzed, in accordance with the predominance of the air (dust) route of transmission of the infection, most often the thoracic form of tularemia was observed, whereby the severity of the course of the disease could vary considerably -- from mild cases with slight (frequently overlooked clinically) involvement of the upper respiratory tract to severe pneumonia. In a small percentage of cases anginal-bubonic, abdominal, ophthalmic-bubonic and other forms of tularemia were noted. The opinion was expressed that in agricultural outbreaks the infection occurs basically by the alimentary route and, correspondingly, in the patients the intestinal form of tularemia is predominant (L. V. Gromashevskiy, 1947). However, this opinion is not shared by the majority of epidemiologists as being contradictory to the factual data (Yu. A. Myasnikov and O. V. Ravdonikas, 1954; I. I. Yelkin, 1959 and others). Epidemiological observations as well as experimental studies of laboratory animals confirm the ease of aspiration infection with minimum doses of the pathogen. On the other hand, it was established experimentally that infection through the intestine is usually achieved only with massive doses of the tularemia microbe, because its penetration into the intestine is prevented by the bactericidal power of the gastric juice (R. A. Savel'yeva, 1956, 1958). Frequent involvement of the respiratory tract in cases of the agricultural type also speaks for an aspiration mechanism of infection.

In view of the fact that the agricultural type of outbreak has been described inadequately in the literature, we are presenting some data below which were collected by the comprehensive tularemia expedition of the IEM imeni Gamaleya during the period from 1946 through 1949 in the southern portion of Moskovskaya Oblast, where this type of disease was predominant.

This expedition was headed by N. G. Olsuf'yev. I. N. Mayskiy, N. P. Naumov, T. N. Dunayeva, V. V. Kucheruk, G. P. Uglovoy, Ye. V. Karasayeva and others, as well as workers in Mikhnevo Tularemia Station A. I. Nikolayeva, P. N. Glagoleva and M. A. Rubina participated in it. During the first two years L. M. Khatenev and his co-workers V. P. Motornaya, S. Gorinshteyn and others also participated. It was determined that on the territory of seven rayons (Mikhnevskiy, Serpukhovskiy, Kashirskiy, Stupinskiy and others) where observations were made natural foci of tularemia of the meadow-field type were of the greatest epidemiological importance; of lesser importance were foci of the soddy-alluvial-boggy (chiefly in the Oka

River Valley) and forest types. During the period from 1938 through 1948 in these rayons 1,057 cases of tularemia were recorded among people. By years the incidence was distributed in the following way: in 1938, 211 persons; in 1942, 367; in 1943, 189; in 1944, 36; in 1945, 136; in 1946, 86; in 1947, one; and in 1948, 31 persons.

Morbidity rate peaks were observed in 1938 and then in 1942-1946 and were most often associated with the delayed winter-spring threshing of cereal crops and other agricultural operations (Figs 42, 43) against the background of a rise in the census of the common vole during these years (Fig 44) and the development of a tularemia epizootic in its population. In the winter of 1937/38 delayed threshing occurred in various kolkhozes of Mikhnevskiy and other rayons and was associated with a very good cereal crop harvest in 1937 with a shortage of threshing machines, whereas in 1942-1944 the delay in threshing was brought about by a shortage of help (the male population had been drafted into the army).

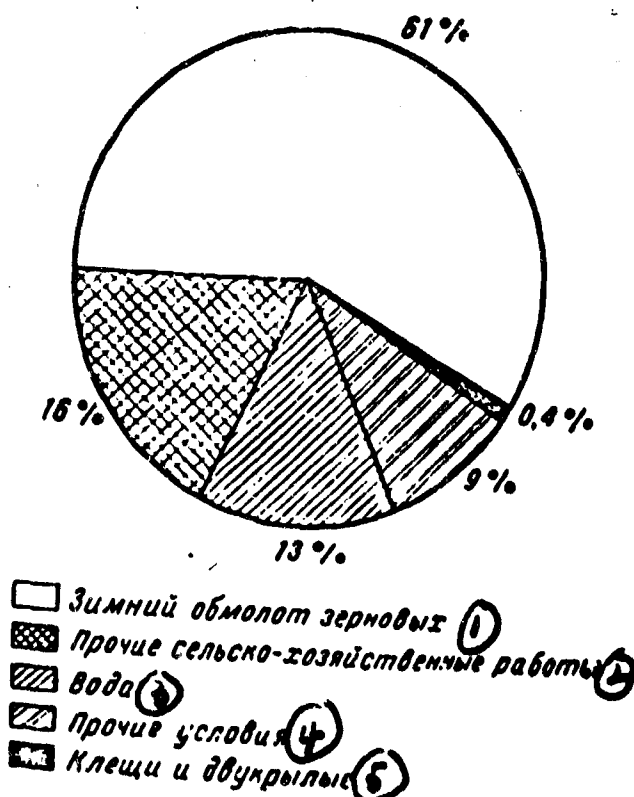


Fig 42. Distribution of Cases of Tularemia in People According to Conditions of Infection and Routes of Transmission. South Moskovskaya Oblast. 1. Winter threshing of cereal crops; 2. Other agricultural operations; 3. Water; 4. Other conditions; 5. Ticks and Diptera.

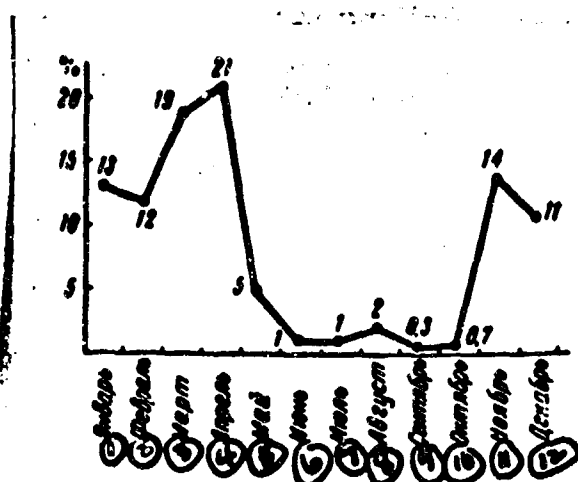


Fig 43. Movement of the Morbidity Rate by Months (in Percentages of the Annual Morbidity Rate). South Moskovskaya Oblast. 1. January; 2. February; 3. March; 4. April; 5. May; 6. June; 7. July; 8. August; 9. September; 10. October; 11. November; 12. December.

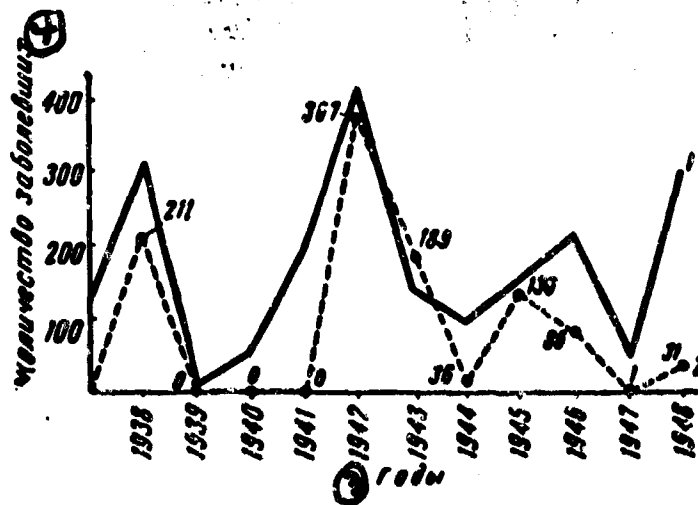


Fig 44. Variations in the Census of Mouse-Like Rodents (1) and Incidence of Tularemia (2) for 11 Years. South Moskovskaya Oblast. 3. Years; 4. No. of cases.

An analysis of the cases shows that those associated with delayed threshing were not the only type of disease of the agricultural type. Sixty-one percent of the cases came from infections during



threshing and 16 percent from other agricultural operations. In this group are included the carting of straw and hay, giving feed to the cattle, sorting potatoes, etc. We should like to note that in 1944, of 12 cases of tularemia from agricultural operations only one case occurred during threshing. In the focus under study 13 percent of the cases occurred from the water route of transmission of the infectious disease; 0.4 percent, from the arthropod-borne route (blood-sucking Diptera, ixodid ticks); and nine percent, under other conditions. Domestic, occupational and other diseases are included among the "other" group.

An investigation of the population in 1946-1948 by means of the performance of an intradermal tularin test and collecting anamnestic data showed that cases registered in a period of 11 years reflect only a small part of the people who recovered from tularemia previously. The actual morbidity rate was much higher. In Mikhnevskiy Rayon, in 26 inhabited places 1,515 adults and 46 school children were investigated selectively; of these, in the first group 21 percent reacted to tularin; in the second, 8.7 percent. In Serpukhovskiy Rayon, in 15 inhabited places, a selective investigation was made of 346 adults and 104 school children; of these, 28.6 percent in the first group reacted to tularin; in the second group, 11.5 percent. The population investigated had never been inoculated against tularemia, and the positive allergic reactions could have occurred only as the result of having had tularemia.

Judging from the anamnestic data, the majority of persons had the disease during the period 1942-1946, and part, in 1938. According to tentative calculations the number of people demonstrated in the investigated inhabited places who had had the disease in retrospect exceeded the number of tularemia patients registered officially during the period from 1938 through 1948 by approximately 10 times. During the investigation it was noted that the segment of the population which had had tularemia varied in the various inhabited places, which was explained chiefly by their being under different landscape conditions and, apparently, by the fact that these inhabited places were different from one another in their work and domestic features. The effect of the latter on the incidence of tularemia is seen through the example of a comparative study of this incidence in two kolkhozes of Serpukhovskiy Rayon located next to each other under the same landscape conditions. It was made clear that in kolkhoz L., where the agricultural operations were carried out in time and the kolkhoz members lived under better monetary circumstances and at a higher cultural level, the incidence of tularemia was much less (12 percent had the disease) than in the poorer M. kolkhoz, where agricultural operations lasted a longer time (68 percent had the disease).

In the study of the focus we had an opportunity to observe a number of small tularemia outbreaks of agricultural type. In one of the kolkhozes of Serpukhovskiy Rayon, where the threshing of cereal crops dragged on until the end of December 1947 cases of tularemia occurred. During the period from the end of December 1947 through March 1948 20 persons had the disease. In some the disease had a lingering character lasting more than three months. In nine patients the local physician made the diagnosis of influenza; in five, pneumonia; in four, malaria; in the rest, no diagnosis was made.

The investigations showed that all 20 persons were sick with tularemia; of these, 13 participated in the threshing; the infection of the others occurred either from giving feed to the cattle (three persons) or under home conditions (four persons), particularly in drying grain at home, during the use of straw for fuel, etc. On the threshing floor where straw-stacks infested with rodents remained after the threshing a tularemia epizootic was found among voles.

In Serpukhovskiy, Mikhnevskiy and other rayons, beginning with 1948, mass inoculations of the population with tularemia vaccine were conducted under the supervision of the expedition, and in 1953 the cases in these regions had been reduced to the level of sporadic cases, although tularemia epizootics among rodents continued to be found from time to time (M. A. Rubina and coauthors, 1955).

Above, we have analyzed cases of tularemia of the agricultural type, the occurrence of which is associated with small mouse-like rodents. This type of disease has so far been recorded only in the European part of the USSR and has not been noted in Western and Eastern Siberia or in Kazakhstan (with the exception of its western portions), which is associated with the absence or low census of the common vole here. However, in Western Siberia and in the north of the European part of the USSR cases of the agricultural type have been noted with infection coming from the water rat in cases where it settles in the ricks and there is a delayed threshing of them. These cases are characteristic only of the natural foci of the soddy-alluvial-boggy type and occur during the autumn-winter months. The mechanism of infection is by aspiration; less often, alimentary or contact (Yu. A. Myasnikov and O. V. Ravdonikas, 1954).

During recent years, in connection with the considerable enlargement and improvement of kolkhozes, the increase in agrotechnics and extensive mechanization of field harvesting operation as well as mass vaccination of the rural population against tularemia cases of the agricultural type in the Soviet Union have been markedly reduced, and threshing-type outbreaks have become a rarity. This gives us reason for the belief that this type of disease will be one of the first to

be eliminated.

**The Domestic (Home) Type.** Cases of tularemia of this type usually accompanied agricultural outbreaks, but they sometimes were observed independently. Underlying them were tularemia epizootics among the small mouse-like rodents, particularly house mice in the presence of a high census of the latter. G. Ya. Sinay and B. V. Voskresenskiy (1943) called this type of case "contact-food", but this term was considered unfortunate and it is no longer used.

The first agricultural outbreak observed in 1933-1934 in Rostovskaya and Stalingradskaya oblasts (see above), occurred simultaneously with a domestic outbreak. Subsequently, the domestic outbreaks which occurred more often as outbreaks mixed with agricultural ones, were noted in Stavropol'skiy and Krasnodarskiy krais, in the Ukraine, in Moldavia, in the central oblasts of the RSFSR and other places (V. N. Ter-Vartanov and coauthors, 1943; I. N. Mayskiy, 1944; G. P. Slavin, 1946; L. V. Gromashevskiy, 1947; N. G. Olsuf'yev and coauthors, 1953; Yu. A. Myasnikov and O. V. Ravdonikas, 1954, and others).

Cases of infection occurring during life at home, in the house or on the homestead are called "diseases of the domestic type". Characteristic of them is a familial incidence, although sometimes only one of the family members can become sick. In the case of familial disease the false impression has been created that the healthy members of the family are infected from those who have been sick previously (L. M. Khatenever, 1946). In the South, the infection is usually brought into the houses (homesteads, workers' settlements) by house mice which emigrate from the fields in the autumn; in the central oblasts a similar role is played by common voles. However, the scale of migration of house mice into the houses is considerably greater than that of voles, which accounted for their leading role as sources of the infectious disease in the case of domestic infections, particularly in the south. Rodents sick with tularemia contaminate surrounding objects, food products, water, forage, etc. by their excretions, and infection of man occurs from these objects as well as from the rodents themselves.

Depending on the size of the territory on which the high rodent census was observed, cases included from several oblasts to one village and even were limited to a single family (Yu. A. Myasnikov and O. V. Ravdonikas, 1954). All ages were equally affected. Sometimes the cases occurred even after the conclusion of the epizootic among rodents (the sorting of potatoes in the potato vault in the spring, the use of the previous winter's straw for heating, etc.). The mechanisms of infection were most varied: aspiration (heating with straw, weeds,

cornhusks, drying and grinding of grain under home conditions, sweeping the floor, giving fodder and bedding to the domestic cattle and poultry, etc.); by contact (with the bodies of mice, bites of mice and cats, etc.); alimentary (contamination of food products and water by rodents, etc.). The morbidity rate was highest from November through February, but sometimes dragged on until June. As an example, we should like to dwell on an outbreak which we studied, observed in 1940 in Stavropol'skiy Kray (I. N. Mayskiy, 1944).

An increased census of mouse-like rodents in the fields was noted in the spring of 1940, but by October-November it was tremendous. The number of holes of common voles at this time reached 60,000-100,000 per hectare (according to the data of N. P. Naumov and the local Plague-Control Station. The tularemia epizootic, developing against the background of an exceptionally high census of small mouse-like rodents, was very intense. Of predominant significance as sources of the infectious disease were house mice, in which from September 1940 through May 1941 at different points in the kray tularemia was found in 193 cases. Simultaneously, the infectious disease was found in common voles, brown rats, wood mice, gray hamsters [*Cricetulus migratorius*], gray hares and other mammals (V. N. Ter-Vartanov and coauthors, 1943).

Observations in the foci of infectious disease which occurred confirmed the exceptionally great epidemiological significance of house mice in the spread of the infectious disease. Having reached a tremendous census by the autumn, the mice, with the advent of the cold, began to migrate and resettle from the open spaces in the workers' settlements and to concentrate in the houses, granaries, warehouses, etc. This led to an increase of the tularemia epizootic in their population, and after this a mass outbreak of tularemia began among people. During the period of their mass migration the mice literally flooded the houses, contaminated not only food products (bread, meat, grain) but also household articles.

Of the other species of rodents in this outbreak a considerable epidemiological role was played by the common vole. According to the data of zoologists, Zakharchenko and Timofeyev, the common vole census amounted to 18-20 percent of that for the house mice. The concentration of voles in the stacks of unthreshed grain, straw-stacks, and places in which cotton was kept created conditions for tularemia infection of persons working in threshing, carting straw and hay, cotton, etc.

The mass multiplication of mouse-like rodents, which reached the scale of a "mouse invasion", and the tremendous number of individuals among them infected with tularemia created an exceedingly

high concentration of the tularemia microbe in the focus, which was spread out over a large territory. Food products -- bread, flour, groats, grain, vegetables, fruit, nuts, etc. -- as well as drinking water were contaminated by the excretions of the rodents and became routes of transmission of the tularemia infection. Considering the richness of the kray in all-possible agricultural products which were sent to all corners of the Soviet Union (particularly grain, hay, cotton, wool, meat, etc.), the real threat of spread of tularemia to territory free of it could be understood. Despite the measures taken to prevent such a transfer of the infection, it did occur in one case: in Baku grain infected with the tularemia microbes was imported. Because of the low census of local rodents (house mice) and measures taken in time for extermination of them the epizootic which began among these animals was quickly eliminated, and tularemia was not observed among the population. However, persons who dissected the rodents and made laboratory examinations became sick.

In the situation which was created in Stavropol'skiy Kray infection of people occurred by different routes: a) direct contact -- with mouse-like rodents and their excretions; b) by mouth -- by contaminated hands or from the consumption of infected food; c) through drinking water; d) through dust -- the aspiration route, from the tilling and processing of cereal and forage products, etc. Along with the infection from small mouse-like rodents in the autumn (September-October) tularemia in four people was noted as the result of bites of pasture ticks (species not determined).

In view of the variety of conditions and modes of infection of people in the same focus different types of outbreaks developed. Not uncommonly, in the same inhabited place it was possible to observe an outbreak of water character associated with the consumption of infected well water for drinking; a case of the hunting-food type in persons engaged in hunting hares; cases among persons participating in the winnowing of grain and, finally, some patients were infected as the result of direct contact with rodents or from the consumption of infected food products. In a number of inhabited places 50-60 percent of the inhabitants were sick with tularemia; thereby, not uncommonly cases began simultaneously in the entire population of a village at intervals of no more than two-three days.

Work in threshing grain and winnowing it, sifting flour, carting straw and hay, which required extensive participation by the population, also created conditions for mass tularemia infection of people. In a number of cases it was very difficult to find out the conditions under which the tularemia infection had occurred, because all factors were present simultaneously: infected water, participation of

the population in agricultural work, in threshing, winnowing grain, etc., infected food, contacts with rodents and contaminated objects.

The highest census of mice and the peak of the epizootic preceded the moment of the highest morbidity rate in the population by several days. Thus, in the village K. the highest number of mice was observed between 10 and 15 November; the greatest number of cases of tularemia in people, between 20 and 25 November. The same picture was also noted in other inhabited places. Such facts have been established also during a similar outbreak in the autumn of 1942 in Stalinskaya Oblast (Fig 45). The maximum morbidity rate for Stavropol'skiy Kray as a whole was noted between 20 and 25 November (Table 24).

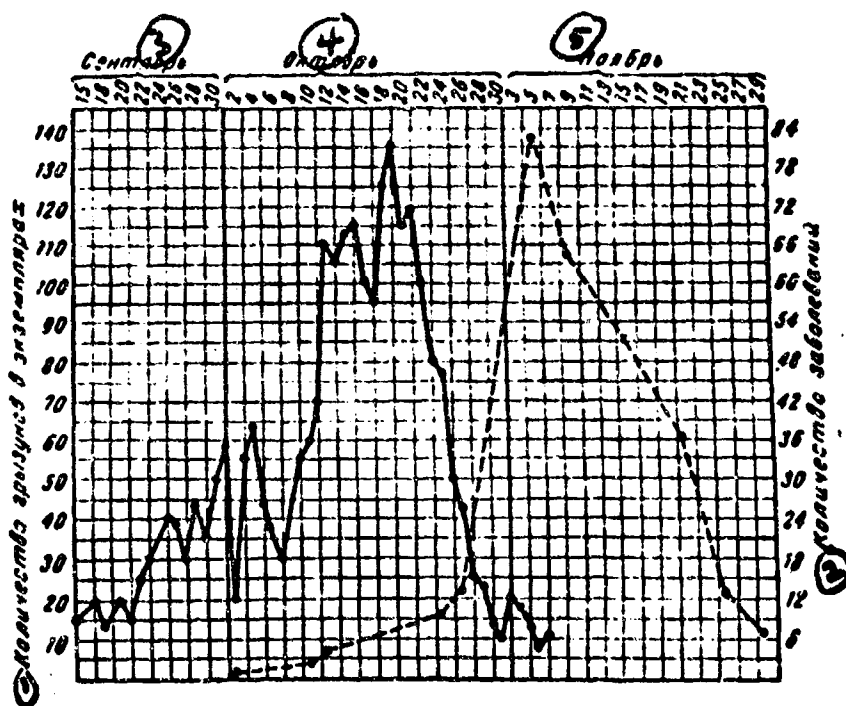


Fig 45. Variations in the Census of Mice in the Houses and the Movement of the Morbidity Rate of Tularemia in People. The solid line shows the number of mice caught per day; the broken line, the number of people who became sick. 1. No. of rodents; 2. No. of cases of disease; 3. September; 4. October; 5. November.

Table 24

Incidence of Tularemia Among People by Five-Day Periods in Stavropol'skiy Kray in 1940

① Месяц	② Число	1-5	6-10	11-15	16-20	21-25	26-30	⑦ Всего
③ Октябрь . . . . .		—	—	1	1	1	1	4
④ Ноябрь . . . . .		11	28	145	1 035	4 106	1 840	7 165
⑤ Декабрь . . . . .		628	388	190	166	204	38	1 614
⑥ Итого . . . . .								8 783

1. Month; 2. Number; 3. October; 4. November; 5. December; 6. Total; 7. Grand total.

Five persons died of tularemia, which amounts to 0.03 percent. It should be noted that along with cases of tularemia medical workers recorded a mass outbreak of influenza during the period of the tularemia outbreak. Thereby, the increase in the number of cases of influenza, just like those of tularemia, reached a maximum during the period between 20 and 25 October. This is apparently explained by the incorrect diagnosis of a large number of cases of tularemia which went under the diagnosis of influenza. With the aim of making the diagnoses more accurate 500 patients with influenza were studied by means of using allergic and serological tests. The specific reactions for tularemia were found positive in 60 percent of the cases.

An analysis of the morbidity rate by sex and age shows evidence to the effect that in the case of domestic infection the incidence of tularemia does not depend on these factors. In the village K. of 352 persons who became sick with tularemia there were 160 men and 192 women. Seventy-one persons under the age of 10 became sick (including two children under the age of one); 71, from 11 to 20; 65, from 21 to 30; 80, from 31 to 40; 42, from 41 to 54; and 23, over the age of 50.

With respect to the clinical picture in this outbreak the pulmonary form amounted to 6.5 percent; the "generalized form", 49.5 percent; the bubonic form, 44 percent; here, the anginal-bubonic

form was observed in 13.5 percent and the ophthalmic-bubonic form, in 0.3 percent. At the present time, it is not possible to break down more accurately the group of patients with the diagnosis "generalized" form of tularemia. In it there could have been cases of abdominal and thoracic forms. Since the occurrence of various clinical forms is associated with the mode of infection it may be stated that in the given outbreak apparently the alimentary route of infection was the main one. At the same time, a great part was played by direct contact with infected substrates as well as by the aspiration route of infection.

In outbreaks of domestic and agricultural types, studied later, a marked predominance of the aspiration mechanism of infection was determined, and in accordance with this, the predominance of the thoracic form of tularemia (N. G. Olsuf'yev and coauthors, 1953).

During a mixed domestic and agricultural outbreak in 1933-1934 (in Rostovskaya Oblast), S. Ya. Kreytser, N. K. Grzhebina and A. S. Kvasinnina (1935) noted cases of tularemia in domestic rabbits and simultaneously in people taking care of them. The authors considered the rabbits the source of infection of people; however, M. F. Shmuter (1955) justifiably doubted this conclusion. In warreners the disease had a course of the anginal-bubonic type, and it is more probable that the infection occurred by the alimentary route from some other source, for example, from water or food products. Under similar epidemiological conditions (that is, during a period of the active winter epizootic among mouse-like rodents and the mixed domestic and agricultural outbreaks in people) M. F. Shmuter also observed (in 1949) cases of tularemia among domestic rabbits on a rabbit breeding farm (infection of them occurred through the feed), but only a single case of tularemia was noted among the service personnel of the farm: a veterinary physician became sick after dissecting a rabbit which had died; the form of disease was ulcerative-bubonic (with the formation of small ulcers on the phalanx of the finger and a corresponding enlargement of the antecubital and axillary lymph nodes).

In the literature there are indications of cases of infection of people with tularemia while taking care of hogs (A. F. Komarova, 1945) or during consumption of their meat as food (V. N. Zil'fyan, 1958), but without bacteriological proof of the fact that these hogs were actually the sources of infection. Finally, in the case of domestic and other outbreaks of tularemia occasional cases in people have been associated with infection from cats (A. F. Komarova, 1945, and others) without a precise indication of the circumstances under which it occurred. Considering the high degree of resistance of cats to tularemia and the extremely low degree of infection of their bodies even when they are infected purposely with massive doses of the tularemia micro-



these cases should be considered mechanical (from bites, scratches, etc.) transmission of the infection by animals who were in contact with sick rodents.

**The Food Product Type.** In this type of outbreak are cases associated with group infection from food products infected somewhere (in the store, storehouse, dairy, dining room, etc.). Bread, milk, pastry, biscuits, etc. contaminated by the excretions of rodents sick with tularemia, usually house mice, can be such food products. Outbreaks of the food product type usually accompanied cases of the domestic and agricultural types, but sometimes they occurred independently. They were first observed by L. M. Khatenever and I. N. Mayskiy in 1940 in Stavropol'skiy Kray (I. N. Mayskiy, 1944). Subsequently, outbreaks of this kind were described by I. R. Drobinskiy (1949), S. P. Karpov and A. D. Lebedev (1949), Yu. A. Myasnikov and O. V. Ravdonikas (1954), M. F. Shmuter and Ya. L. Svitsent (1958) and others in observations made in the central zone of the European part of the RSFSR (Tul'skaya Oblast), in the Ukraine (Khar'kov), in West Siberia (Kemerovskaya Oblast) and other places. The period in which cases of this type occurred was December through June. In their clinical picture there was a marked predominance of the anginal-bubonic form of tularemia, which corresponded to the alimentary route of infection. The outbreaks were local usually, numbering from several to tens of cases, and ended immediately after the utilization, destruction or disinfection of the infected food product. I. N. Mayskiy and L. M. Khatenever noted cases of tularemia in people who chewed on sunflower seeds which had been damaged and contaminated by mice. According to the data of M. F. Shmuter and Ya. L. Svitsent (1958), in Khar'kov in 1948 a small tularemia outbreak was demonstrated associated with the consumption of food products bought in the market (milk or sugar). Over a period of five days 24 persons became sick; of these, 17 had the anginal-bubonic form and seven had "localizations of the lesions in the internal organs" (abdominal form?). During this period, in the locality to which the food products had been brought, an active tularemia epizootic was noted among mouse-like rodents. The tularemia outbreak in Kemerovskaya Oblast described by S. P. Karpov and A. D. Lebedev (1949) deserves attention; it was associated with the consumption of infected milk which came to one of the public dining rooms from a dairy. The outbreak was observed in October; in seven days 44 persons became sick, and in all the anginal-bubonic form of tularemia was diagnosed. The infection of the milk occurred from rodents which lived on the premises of the dairy and in the dining room, which was confirmed by isolation of cultures of tularemia pathogen from rodents caught (house mice and brown rats).

The prophylaxis of this type of case should be based primarily on strict observance of rules of sanitation in the storage and consumption of food products.

**Food-Industrial Type.** Cases of this kind occurred at enterprises of the food industry during the primary processing of agricultural food products as well as during the slaughtering and dressing of certain kinds of meat which had come from a locality unfavorable with respect to tularemia. Because the food products (or animals) were sometimes brought in from far, and the enterprises in a number of cases were located in cities, these cases usually occurred unexpectedly in the presence of complete well-being of the surrounding territory with respect to tularemia. A lack of correlation of the places at which cases of the food-industrial type occurred with the natural foci of tularemia as well as the strictly occupational nature of these cases distinguish their epidemiology and prophylaxis sharply from outbreaks of other types.

The food-industrial type of tularemia was comparatively recently distinguished as an independent one (Yu. A. Myasnikov, 1955). It should be noted that outbreaks of this type were first observed in the USSR almost 30 years ago (I. F. Berezin, 1931), but they were not correctly classified. Cases of this type can be divided into two groups according to their epidemiological characteristics; of these, the first group is associated with the reprocessing of agricultural products; the second, with the slaughter of animals and dressing of the meat.

**Infection from the Reprocessing of Agricultural Products.** In 1940, I. N. Mayskiy and L. M. Khatcnever, in one of the cities of the North Caucasus, observed cases of tularemia among workers in mills and grain elevators, who were engaged in receiving and processing the grain which came in. In the subsequent years, Yu. A. Myasnikov (1955), A. P. Levchenko (1955) and M. F. Shmuter (1959) described cases of tularemia among the workers in sugar factories. These cases were observed in the winter of 1948-1949 on the territory of the Ukraine as well as in Voronezhskaya, Kurskaya, Tambovskaya and Tul'skaya oblasts of the RSFSR. Tularemia among the workers in sugar factories located in certain oblasts of the Ukraine and RSFSR occurred in 1946, but no epidemiological analysis was made at that time. According to the data of the authors mentioned, cases of tularemia among the workers of sugar factories were observed in the autumn-winter. They began in November, reached the maximum in December (or January), coinciding with the period of most active processing of sugar beets, whereas in February, March or April the cases were isolated ones.

M. F. Shmuter (1959) points out that in the winter of 1948-

1949 in six oblasts of the UkrSSR more than 3600 persons became sick with tularemia at sugar factories. The infection occurred from sugar beets coming into the plants with signs of contamination and damage by rodents (house mice, voles) from regions where an active tularemia epizootic had been observed among small mouse-like rodents. In these regions considerable outbreaks of the agricultural and domestic types were noted. Infection of the sugar beets was proved by the isolation (by a biological test) of cultures of the tularemia pathogen from washings taken from it (Yu. A. Myasnikov; M. F. Shmuter). The low temperatures of the autumn-winter months, at which time the sugar beets were transported to the plants, contributed to the prolonged preservation of the tularemia pathogen on the surface of the beet. At the sugar factories cases of tularemia occurred first among workers in the beet-processing shop, where the sugar beets are washed and cut. In a number of cases almost all the workers in this shop became sick with tularemia. A large number of cases was also noted among persons who worked in other shops and who frequently visited the beet-processing shop by virtue of the nature of their work activity, specifically workers in the repair and power shop and laboratory workers. A considerable number of cases was also noted in the juice-purification shop, chiefly among persons working next to the beet-processing shop, for example, among laundresses who cleaned the napkins for the filter presses. As far as the workers of the other shops are concerned, particularly in the auxiliary shops (the products shop, boiler room, transportation room, office and others), the morbidity rate among them was several times lower than among the workers in the beet-processing and repair-power shops as well as the workers of the laboratory. In some of the sugar factories up to 23 percent of the personnel became sick. The number of patients corresponded approximately in age and sex to the age and sex composition of the workers in the corresponding shops. The aspiration route of infection was predominant from the inhalation of small droplets of infected water which were present in abundance in the suspended state in the air of the beet-processing shop. Correspondingly, 96-100 percent of the patients had "tularemia of the internal organs", including the pulmonary form which was observed in 34.2 percent of them (Yu. A. Myasnikov). In part of the cases the alimentary route of infection was noted, for example, Yu. A. Myasnikov points out that the abdominal form of tularemia was observed in 1.9 percent of the patients; the anginal-bubonic form, in 1.9 percent. M. F. Shmuter directs attention to the fact that among workers in the beet silo in contact with sugar beets before they came to the factory there were practically no cases of disease, which the author considers indirect confirmation of the aspiration infection of people during

the washing of the sugar beets at the factory.

Infection from the Slaughter of Animals and Dressing the Meat. I. F. Berezin (1931, 1934) describes two tularemia outbreaks observed in the spring of 1930 and 1931 at a canning factory in Kurgan (West Siberia) during the preparation of hare meat for canning. Before dressing the hare carcasses were kept in the frozen form at a temperature down to  $-30^{\circ}$ . In all, more than 100 persons became sick. The patients had the bubonic form of tularemia in which the bubo was located in the axilla or in the area of the elbow joint, which clearly indicated the contact mechanism of infection. In two cases an anginal-bubonic form of tularemia was observed. Only workers in the batching department who were in contact with the carcasses or meat of the hares were infected. There is every reason to consider these outbreaks of the food-industrial type rather than of the hare (hunting-food type according to the current classification), as had been done previously.

M. F. Shmuter (1955) noted two cases of tularemia in people during the dressing of carcasses of hares at one of the meat combines of the Ukraine. The infection occurred at the end of March - beginning of April in a department in which the rabbits were slaughtered and their carcasses dressed. The rabbits came to the meat combine from areas unfavorable with respect to tularemia including from the rayon in which the author had found cases of tularemia in rabbits in one of the rabbit breeding farms during a period of an active tularemia epizootic among small mouse-like rodents.

We also classify classes observed at meat combines during the slaughter of domestic ungulates under the heading of the food-industrial type. In the USSR such cases were described for the first time by R. Ya. Chernin (1953), associating infection of people with the presence of a large number of ixodid ticks on cattle coming to the meat combine, from which the author isolated the tularemia pathogen by the biological test. R. Ya. Chernin believes that infection of people occurred at the time the cattle were slaughtered both from the ticks and from the excretions, which were found in large quantity in the fur of the animals being killed. In sick people the ulcerative-bubonic form of tularemia was observed with a localization of the primary lesion on the hands. The cases were observed in the autumn.

In October-November 1954, in Leninakan (Armenia) at a meat-canning combine a tularemia outbreak occurred associated with the slaughter of sheep (S. M. Smirnov, 1956; M. P. Tereshchenko and coauthors, 1956). N. N. Zhukov-Vereshnikov, whose materials we are using for the description of this outbreak, took a direct part in the elimination of it. Cases of tularemia in sheep have been recorded repeatedly abroad (United States); they have also been described in the

USSR (see Chapters II and V); however, cases of infection of people from sick sheep or their meat have not been found. American and Canadian investigators have repeatedly observed cases of infection of people taking care of sheep and particularly during the shearing of the animals on which there were ticks and their excretions infected by the tularemia pathogen.

Cases of tularemia among people in the Leninakan meat-canning combine occurred during a period of mass slaughter of sheep, chiefly among workers in the slaughterhouse and byproducts shop (in both shops the number of cases amounted to 79 percent of the total cases of tularemia for the combine). Chiefly persons who cut sheep or dressed the fresh meat became sick. In the slaughterhouse 51 percent of the workers became sick with tularemia; in the byproducts shop, 41 percent; in the hide-salting shop, 31 percent, etc. In the canning and sausage shops, where the meat was brought for processing after a 24-hour refrigeration, only occasional cases of the disease were observed, which indicated the low degree of infectivity of such meat. In 62 percent of the people who became sick the bubonic form of tularemia was noted, whereby in the majority the buboes were localized in the axilla, which indicated contact infection through the upper extremities. Numerous cuts, abrasions and other minor injuries on the hands of the workers contributed to the penetration of the pathogen through the skin. Among the bubonic forms several cases of anginal-bubonic and ophthalmic-bubonic forms of tularemia were noted. In 33 percent of the patients there were no external inflammatory changes (including buboes), whereby in one-third of the cases involvement of the respiratory tract was noted, which could serve as an indication of the aspiration route of transmission of the infectious disease. In the shops of the combine the floors and panels were washed by means of firehoses and under the influence of the strong stream of water a mist was created in the air made of water-bearing dust containing an admixture of blood of the killed animals. The abdominal form of tularemia was observed in two patients.

On examination of sheep it was possible to isolate the tularemia pathogen from the organs in the case of three animals by the biological test (chiefly, from the lymph nodes). Numerous cultures were also isolated from *Haemaphysalis oxophila* ticks found in large numbers on the sheep. Infection of the sheep evidently occurred from ticks which had crawled onto the animals during their drive through a locality in which there were natural foci of the disease.

In the outbreak described it was determined accurately that sheep brought from areas unfavorable with respect to tularemia were the sources of cases of tularemia in workers of the meat combine

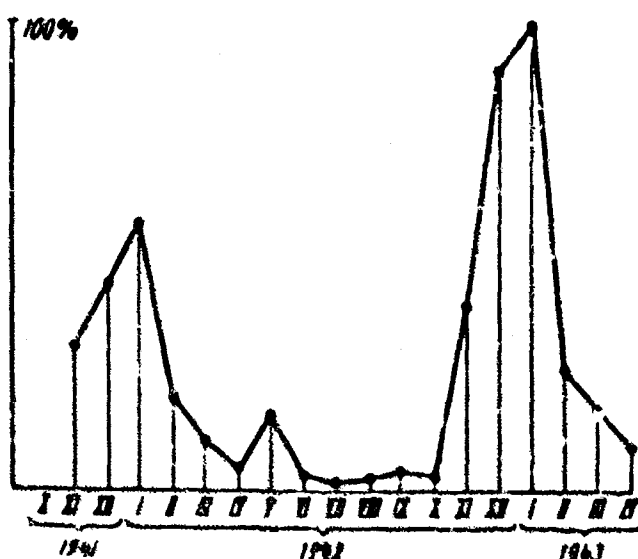
and that infection occurred at the time of slaughtering the sheep, both from the animals themselves and from ticks parasitic on them. During the first few days of the outbreak which had begun, when the diagnosis of the disease and the causes of the sickness had not yet been established, the meat and byproducts from the killed sheep were delivered to stores and public dining rooms in the chilled or frozen form. A total of one case of tularemia, occurring from the dressing of ram heads which had been purchased, was detected. People were not found to be infected from the meat. Nor were there any cases found among workers in the tanning plant to which the hides taken from the sheep were brought (S. M. Smirnov, 1956).

**Trench Type.** Cases of tularemia of this type occurred under conditions at the front, when ditches, trenches, dugouts, blindages, etc. were used during the period of military operations for housing the troops. The occurrence of disease was associated with the mass settlement of these structures by small mouse-like rodents sick with tularemia (house mouse, common vole and others). Outbreaks of this kind attracted attention and were studied in detail during the years of the Second World War (G. P. Rudnev, 1943; I. N. Mayskiy, 1944; A. I. Volkov, 1944, and others). A number of authors believes that the disease observed during the First World War during the positional warfare of the troops on the Russo-German Front and described under the name of "Volhynia" or "trench" fever was, in part of the cases, tularemia (L. V. Gromashevskiy, 1947; A. I. Volkov, 1948, and others). The type of case (outbreak) being analyzed here was described in detail by K. F. Akinfiyev (1955) and G. P. Rudnev (1955), to whose works we refer the interested reader. Below, we shall dwell only on the main characteristics of this type of disease. In their epidemiological and epizootological characteristics the trench outbreaks were very similar to the domestic ones.

During the years of the Second World War the basis for the appearance of a large number of rodents in the area of military operations was a marked disturbance of agricultural operations, particularly a poor harvest, untimely plowing of the fields, etc. In the fields the ricks remained unthreshed; straw, hay lay unharvested; in the plowlands and in the inhabited places weeds grew, etc. All this created particularly favorable conditions for the mass multiplication of common voles, house mice and other rodents. With the advent of the cold weather a concentration of rodents was noted in the trenches, blindages and other structures. In large measure the rodents fell into the open trenches and passages involuntarily during their movements, because these structures became a kind of "gutter trap" for them. For the purpose of heating the blindages as well as for litter

and for stuffing mattresses, extensive use was made of straw from straw-stacks infested with rodents, which was also of epidemiological importance.

The greatest number of cases among people was observed when the positions were located in an open locality among fields and meadows. The maximum morbidity rate was noted in November-December-January (Fig 46) and then in March-April during the period of the snow thaw (or in May) a second wave of disease was observed (G. P. Rudnev, 1943; K. F. Akinfiyev, 1955). In the dugouts and blindages constructed in the forest, cases of tularemia among people were rare. This is explained by the fact that other species of rodents live in the forest and there are few conditions there which contribute to the development of an active tularemia epizootic among them in the wintertime.



of dust from the use of straw contaminated with sick rodent excretions for litter. In accordance with this, there was a predominance of the pulmonary (thoracic) form (G. P. Rudnev, 1943, 1955). Frequently, infection by the oral route was observed as the result of consumption of food products and water infected by sick rodents. In such cases the anginal-bubonic and abdominal forms developed. Cases of infection as the result of direct contact of man (hands, legs) with sick or dead rodents were comparatively rare. Cases of infection were noted through cuts in the hands or legs made by infected straw (from pulling straw out of straw-stacks and stuffing mattresses, using straw for packing in boots or valenki [felt boots]).

The mortality rate from tularemia under the epidemiological conditions being considered amounted to 0.17-0.3 percent (G. P. Rudnev, 1955).

Water-borne and food outbreaks were observed together with the trench type in the theater of military operations.

#### Interrelationship of Different Types of the Disease

Materials accumulated in the USSR concerning the movement of cases of tularemia and their epidemiology are evidence to the effect that for more than 30 years (from 1926 through 1959) tularemia in people has occurred at different periods not only with different degrees of intensity (see Chapter I), but also with different quantitative relationships between the separate epidemiological types of disease (outbreaks).

During the first few years of study of tularemia it was believed that outbreaks of the occupational type, associated with the water rat, were the main ones. The large-scale occupation revolving about this animal, which occurred in 1927-1928 simultaneously in many places of the USSR, contributed to the occurrence of a large number of cases of tularemia. However, even during this period, which undoubtedly coincided with a rise in the water rat census, arthropod-borne tularemia outbreaks appeared to a notable extent (Astrakhan-skaya, Voronezhskaya and Saratovskaya oblasts, South Kazakhstan, West Siberia, and Yakutiya), which indicates activation of tularemia foci of the soddy-alluvial-boggy type during these years. During the same period cases of the industrial type were noted for the first time. Possibly at that time also cases of other types occurred but they were few and went under different diagnoses. In any case, any considerable outbreak the occurrence of which was associated with small mouse-like rodents would hardly have remained unnoticed during this period. Generally, before 1933 cases of tularemia were recorded every year



in comparatively small numbers and in a limited number of oblasts.

In 1933 and then in 1938 and 1940 large tularemia outbreaks occurring in various places of the European portion of the USSR (North Caucasus, southeast, center) against the background of mass multiplication of small mouse-like rodents attracted attention. During the course of study of these outbreaks the important epidemiological role of house mice and of common voles was proved for the first time, and new types of outbreaks were established -- agricultural, domestic and water-borne. During this period, in various places of the USSR occupational [water rat] and arthropod-borne outbreaks were also observed, and cases of hunting-food infections (from hares) were described for the first time. With respect to the number of cases, arthropod-borne, occupational and other outbreaks were less than those of the agricultural and domestic types.

During the years of the Second World War, when in a number of oblasts, particularly those under temporary occupation, disorders in the management of agriculture led to the mass appearance of mouse-like rodents and the development of an active tularemia epizootic among them, agricultural, domestic and water-borne outbreaks were predominant, distinguished by their mass nature and the variety of routes of transmission of this infectious disease. The characteristic features of wartime and the unique way of life of the population during this period led to the occurrence of a special type of outbreak which we called "trench". These outbreaks were distinguished by certain rules and regulations in the spread of the infection and required special prophylactic measures.

During the first post-War years considerable tularemia outbreaks continued to be observed but they were of a most varied nature. Along with agricultural and domestic outbreaks, in places (for example, in West Siberia) considerable arthropod-borne outbreaks were observed. Outbreaks of the previously little-known industrial type were also noted. During these years mass inoculations of the population with living tularemia vaccine developed by N. A. Gayskiy and B. Ya. El'bert (see Chapter X) were begun with the aim of prophylaxis of tularemia in many places in the USSR. As a result of the inoculations cases of tularemia began to decrease sharply. In the period under analysis (as well as later) in various regions which were not completely covered by the inoculations an investigation of the rural population was made with the aim of determining the percentage of people who had had tularemia. For the purpose of retrospective diagnosis the intradermal tularin test was used which was supplemented in a number of cases by the agglutination test. In foci of the meadow-field type in the south of Moskovskaya Oblast 21-28 percent of the people

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were found to have had tularemia (chiefly during the years of the Second World War) among the adult population; in Tul'skaya Oblast, three-15 percent (Yu. A. Myasnikov, 1955); in foci of the soddy-alluvial-boggy type on the territory of the Volga' Akhtubinsk River Valley within the limits of Stalingradskaya Oblast, three-six percent (V. P. Borodin, 1958); in foci of the foothill-brook type in Staro-Bardinskiy Rayon of Altayskiy Kray, 11-71 percent (G. P. Uglovoy), etc. These data indicate the considerable spread of cases of tularemia among people in the past in the region of active natural foci of this infectious disease.

Study of the epidemiology of tularemia shows that the nature of the cases of tularemia changes in certain periods in accordance with the living conditions. Here, in full measure, the principle of the effect of social factors on the epidemic process is confirmed. Thus, while in 1945 and 1946 96.1 percent of the entire tularemia morbidity for the RSFSR came about in the agricultural, domestic and other outbreaks the occurrence of which was associated with small mouse-like rodents, in 1956 outbreaks of this nature amounted to 19.2 percent for the RSFSR; in 1957, 3.5 percent. Undoubtedly, in addition to vaccination, a marked reduction in the census of small rodents through improvement in the management of agriculture had an influence in reducing the morbidity rate from these types. Against the background of a general marked reduction of cases of tularemia in people in the past 10 years an increase in the proportion of arthropod-borne outbreaks is characteristic. The incidence of tularemia of the arthropod-borne type for the RSFSR in 1956 amounted to 43.2 percent; in 1957, 84 percent. Changes of this kind in the nature of the epidemic outbreaks occurred in other republics -- in the UkrSSR and KazSSR. In these republics, in 1956, 46 percent of the tularemia morbidity rate was from the arthropod-borne type; 11.6 percent from the agricultural type; and 33.5 percent from the water-borne type. As a whole, in the USSR in 1956 and 1957 the maximum number of cases of tularemia occurred in the summer months which, as is well known, is characteristic of infections associated with the transmission of the disease by blood-sucking Diptera or through the water of open water bodies. Of the entire morbidity from tularemia 7.6 occurs in the autumn-winter, at which time the disease is associated with an epizootic in the small mouse-like rodents. Everything stated is evidence to the effect that in accordance with the conditions created cases of tularemia among the population can be expressed in the form of outbreaks of different characters, distinguished from one another by the distinctiveness of routes of transmission of the infectious disease, mechanisms of infection as well as by the clinical course of the disease.

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The marked reduction in the number of cases of tularemia achieved in the USSR in the past decade was the result of extensive vaccination of the population conducted in combination with other measures, directed at the elimination of rodents and ticks.

### Conclusion

Investigations made by Soviet authors have made it possible to study the characteristics of tularemia epidemiology in the USSR completely and thoroughly, to learn the basic rules and regulations of the epidemic process, and work out a harmonious system of prophylactic measures. An important achievement was the development of the classification of epidemiological types of the disease (outbreaks), on the basis of which it was possible to plan and carry out the prophylaxis of tularemia in a differentiated manner.

We have come to a period in which the problem of eliminating tularemia in people in the USSR has been posed; this can be done, because at the present time, in addition to the great experience in control of tularemia and more thorough knowledge of its epidemiological characteristics, such a powerful prophylactic measure exists as living tularemia vaccine, inoculation with which affords protection against the infectious disease for a number of years. By combining careful vaccination with other measures it is possible to eliminate completely the cases of tularemia in people in the Soviet Union.

## Chapter IX

### Immunology of Tularemia

#### General Comments

Soviet investigators of tularemia, following the best traditions of Russian microbiology, have from the very beginning of the study of this infectious disease begun to work out problems of its immunology in their inseparable relation with pathology. Particular note should be made of B. Ya. El'bert and N. A. Gayskiy, who through their investigations laid sound bases of theoretical and practical immunology of tularemia. The investigations of I. N. Mayskiy, generalized on in his book Immunology of Tularemia as well as the doctoral dissertations of I. S. Tinker, A. V. Mashkov and V. A. Yudenich, represent an important contribution to the study of immunity in tularemia. We should also like to mention the earlier workers of L. M. Khatenever, G. Ya. Sinay, A. A. Miller and co-workers, N. K. Vereninova, N. D. Altareva and others. Abroad, problems of immunology of tularemia were studied by Francis, Foshay, Downs, Larson, Kudo and others.

The existence of good laboratory models made it possible to study extensively the problems of tularemia immunology experimentally, whereby differences in the infectious sensitivity and immunizability (for the substantiation of these terms see Chapter IV) of the species of laboratory animals used made it possible to conduct investigations from a comparative aspect. In the present Chapter both the data and the literature and materials which our laboratory has at its disposal which have only been partly published are generalized on.

We shall touch only on the rules and regulations of acquired immunity, discussing them chiefly on the basis of experimental studies. Data on vaccination of people, including the organization of inoculations and their effectiveness under practical conditions, have been relegated to a separate chapter, X.

In working out problems of tularemia immunology the principle advanced by B. Ya. El'bert and N. A. Gayskiy (1941) that two different antigenic substances (H and O) are present in the tularemia microbe and that the virulent and immunogenic properties of the cell are associated with only one of these antigens (H), which is lost during the attenuation process, has proved to be very fruitful. By the same token, the intermediate position of vaccine cultures was established in the series of variations of the tularemia bacteria from the

original S virulent form to the R variant obtained on synthetic nutrient media which is completely devoid of virulence and immunogenicity. These ideas about the antigenic structure of the tularemia microbe and its variability have been confirmed completely in our studies done in conjunction with O. S. Yemel'yanova (1958), but El'bert's and Gay-skiy's H antigen was considered a Vi sheath antigen. The virulent S strains, which are the carriers of the ViO antigenic substances, represent strong stimuli and are most active in an immunogenic respect. Vaccine SR cultures, which possess residual virulence, are stimuli of moderate strength. In the antigenic composition of these cultures a reduction is noted in the quantity of sheath (Vi) substances, but qualitatively it is the same, and this assures immunogenicity of the vaccine cultures. As we shall see below, these cultures of different degrees of virulence exert different effects, as seen by the results, on the bodies of animals with different sensitivities to tularemia infection. Finally, avirulent R strains, which are the carriers of the O antigen only, do not have an immunogenic effect against S cultures, which is perfectly natural because of the great differences in their antigenic structures (for more details see Chapter III).

Immunological experiments on laboratory animals, particularly white mice and guinea pigs, are usually accompanied by a check of the strength of immunity in them by infection with many lethal doses of a fully virulent culture. In view of the special sensitivity of these animals to tularemia such a check makes it possible to obtain particularly convincing results. The immunological reactions are used only as indirect indices of immunity.

The requirements for the virulence of a strain by means of which the immunity is being checked in experimental animals should be just as strict as in the investigations of experimental pathology or epizootology of tularemia; otherwise, the results may be very much distorted and may not correspond to the natural conditions of infection.

In a specially performed experiment O. S. Yemel'yanova found out that in checking the immunity of white mice immunized with relatively weak vaccine cultures a dose of 1,000 MLD of a fully virulent strain (MLD = 0.1 microbe) is not equivalent to 1,000 MLD of a partially attenuated strain (MLD = 10 microbes). In the former case, that is, after infection with 100 microbes, only 14 percent of the mice survived; in the latter case, 61 percent survived after infection with 10,000 microbes. Similar results were obtained by I. N. Mayskiy (1953), also in experiments on mice.

In the light of these data we should have a critical attitude toward the investigations of those authors (Kudo, 1934; G. Ya. Sinay, 1935, and others), who used quite virulent strains of tularemia bacteria

for the purpose of checking immunity in experimental animals. In the experiments of the workers in our laboratory O. S. Yemel'yanova, T. N. Dunayeva, T. A. Kalitina, R. A. Savel'yeva, G. P. Uglovoy, K. N. Shlygina and L. S. Matveyets, whose data have been used for writing this Chapter, only completely virulent strains were used for the control infection of animals, in the majority of cases strains 503 or 9, the MLCD of which amounted to a total of one microbe according to the bacterial GKI standard for guinea pigs after subcutaneous injection. The characteristics of these strains are given in Chapter III.

In immunological experiments special attention should also be directed to the quality of experimental animals, the conditions under which they are kept and fed, which was specially advised by N. A. Gayskiy (1948). He pointed out, for example, that the best time for experiments in the immunization of guinea pigs is the beginning of the autumn and the end of the summer because of the existence of fresh vegetables then.

### Active Immunity

#### Immunity from Having Had Tularemia

**Immunity in Animals which Have Had the Disease.** The experiments presented on white rats and domestic rabbits in Chapter IV show that in animals infected with sublethal doses of a virulent strain an immunity is created during the course of the disease under the influence of which the infectious process weakens and clinical recovery of the animal occurs.

In animals which have recovered from the sickness, beginning with the second or third week, the immunity reactions reach their maximum degree of expression, while between the fourth and six weeks the majority of animals eliminates tularemia bacteria completely, and immunity then is preserved for a long time as a sterile or post-infectious type. (This conclusion is based on the negative results of bacteriological and biological (with the use of white mice for passages, studies of tissues and organs of experimental animals). The presence and stability of the immunity are proved by the fact that the animals do not die after being injected with one or several (and sometimes even many) full lethal doses of the virulent strain.

B. Ya. El'bert and N. A. Gayskiy (1941) note that after the administration of a massive dose of virulent tularemia bacteria (a piece of spleen of a white mouse which died of tularemia) intraconjunctivally into rabbits the local and general reactions were moderate and brief (two-three days), were not accompanied by a rise in the agglutination

titer of the serum and were considered allergic by the authors. In the control (infected for the first time) animals vigorous inflammatory phenomena were observed for the first time in the infected eye, a prolonged febrile reaction and the formation of antibodies. The authors note that in rabbits which have had the disease the condition of increased resistance to reinfection was followed for three-six months (the observation period).

In guinea pigs and white mice, because of their great sensitivity to tularemia, it is possible to study immunity after infection with a fully virulent strain only if in certain stages of the disease they are given treatment with streptomycin or other antibiotics which eliminate the infectious process.

In guinea pigs, the treatment of which was begun at the height of development of the disease, a very strong and prolonged immunity to reinfection is observed after recovery. The great majority of cured guinea pigs is sterile with respect to the tularemia microbe, which is evidenced by negative results of studies of their organs with the use of the biological test. In them, the immunity, therefore, is post-infectious. Similar results have been obtained in experiments on white rats infected with a massive dose of a virulent tularemia culture with subsequent treatment with streptomycin (N. G. Olsuf'yev and co-authors, 1957). With the early treatment of guinea pigs the formation of immunity in them can be suppressed and to a greater degree the earlier treatment is begun and the more vigorously it is conducted (Ye. M. Tsvetkova, 1951, 1953). In experiments clarifying the preventive effect of streptomycin in tularemia it was established that by means of the selection of appropriate doses of a virulent culture as well as the doses of the antibiotic and the duration of its application an immunizatory process can be produced in the guinea pig organisms without any particular manifestations of disease, whereby the strength of the immunity which develops is adequate to the dose of culture administered. In guinea pigs immunized by this method active immunological reactions and a resistance to infection with 100 MLCD of a virulent culture were found (Ye. M. Tsvetkova, R. A. Savel'yeva and G. P. Uglovoy).

The experiments presented on guinea pigs and white mice repudiate the opinion of N. A. Gayskiy (1946, 1948) of the absence of immunizatory properties with respect to these species of animals in the highly virulent strains of tularemia bacteria. This opinion was based on the fact that after the administration of minimum (sublethal) doses of a fully virulent strain to white mice and guinea pigs immunity could not be found then in the surviving animals to a subsequent infection with several doses of the same strain known to be lethal. On the

basis of these data N. A. Gayskiy believed that immunogenicity of the tularemia pathogen is not connected with virulence and that "only with a loss of virulence does the tularemia microbe show immunogenic properties characteristic of it" (N. A. Gayskiy, 1948). However, the experiments of N. A. Gayskiy have an entirely different explanation. They only prove the tremendous infectious sensitivity of guinea pigs and white mice to tularemia, as the result of which the minimum infective dose (MID) and the minimum lethal dose (MLD) of the virulent strain for them are practically the same (see Chapter IV). In guinea pigs and white mice and, with the use of a larger infective dose, in white rats the acutely developing infectious process completely suppresses the ability of the body to elaborate immunity. However, as soon as the infectious process is temporarily stopped, for example, by the administration of streptomycin, the immunity develops, and the course of the disease is changed. It has been shown very distinctly that in the animals which recover the immunity created, given adequate strength of it, leads to a purification of the organism [with respect to tularemia]. Cases of chronic bacterial carriage indicate the fact that the defense mechanisms are inadequate for ridding the tularemia bacteria from the body for some reasons or others. However, these cases are not characteristic and do not constitute proof of the lack of sterility of the immunity in tularemia.

**Immunity in People who Have Had the Disease.** Numerous observations on people who have recovered from tularemia, chiefly on those working in tularemia laboratories, have shown that after having the disease an immunity of high strength is found in the body which is perfectly adequate to prevent reinfection under ordinary conditions of contact with infectious material. An abundance of data attests to the fact that in persons who have recovered the high-strength immunity acquired is preserved for many years, and in a number of cases, apparently, for life.

N. A. Gayskiy (1943), after recovering from tularemia, was twice subjected to an accidental infection with a virulent tularemia culture in the laboratory: the first time after three months and the second time, after three years. In the first case the pathogen entered his eye; in the second, the infection occurred as the result of inhalation of tularemia bacteria during an experiment with spraying a culture. The author considered pathological phenomena which occurred in both cases an allergic reaction. L. M. Khatenever (1943) describes very convincing cases in which a stable immunity appeared in persons who had had tularemia and who then were in contact with the infection in natural foci during work in the water rat industry. Francic (1929) notes that of 200 persons who suffered from tularemia none became



sick a second time. Six workers in our laboratory (including the author of these lines) have, after recovering from tularemia, been working steadily with the tularemia pathogen for many years (some persons as long as 25 years), and immunity in them continues to be preserved on a level adequate for preventing the disease under ordinary conditions of contact with the infection. V.D., who had had tularemia 10 years ago and then had no contact with the pathogen of this infectious disease was accepted for work in the laboratory. After checking his allergic skin reactivity, which was found to be well expressed, he was permitted to work with infectious material without restriction, and no harmful consequences to his health have been noted for more than eight years of his work in the laboratory following this. The data presented become particularly convincing if we keep in mind the fact that under the same working conditions non-immune persons have inevitably become infected and sick with tularemia (see Chapter I).

For the long period of existence of the laboratory we have had isolated cases of accidents in working with a virulent culture, where the pathogen entered the eyes of various workers (who previously suffered from tularemia). The inflammatory response which occurred, local or general, usually did not exceed the reaction described by N. A. Gayukiy, with the exception of one case where it was necessary to resort to streptomycin administration. In this case, the patient, aside from redness and edema of the conjunctivae (the specificity of these inflammatory changes was proved by the isolation (through a biological test) of a culture of the tularemia pathogen from the conjunctival exudate), noted enlargement and pain in the anterior auricular lymph nodes, malaise, elevation of body temperature, etc. after several days. After several injections of streptomycin these phenomena rapidly disappeared.

In performing experiments on aspiration infection of laboratory animals under conditions in which entrance of the bacteria into the respiratory tract of the experimenter was not completely prevented, despite the use of a cotton-gauze mask, we were able to note various consequences depending on the dose with which the experimenter worked.

In those persons who had recovered (or who had been vaccinated), in working with small doses either there were no consequences or signs of allergy lasting one-two days were noted which were expressed in an elevation of temperature, headache, weakness, and a poor feeling of well-being. However, after performing the experiment with a large dose both laboratory workers participating in the work (one had had tularemia in the past; the other had been vaccinated) became sick with tularemia, despite the fact that they had worked in cotton-gauze masks.

In both cases the disease began several hours after the conclusion of the experiment, was accompanied by high temperature (Fig 67), severe headache, weakness, and profuse perspiration. In the vaccinated person the disease had a more severe course than in the one who had had tularemia previously. Because the disease continued in subsequent days and because retrosternal pains and a cough were superimposed on the phenomena described streptomycin therapy had to be used, which eliminated the fever. On the seventh-10th day of the disease an opacification of the lung roots and an accentuation of the pulmonary markings were found on fluoroscopy of the chest in both cases. The specificity of the disease was proved by the marked increase in agglutinins to the tularemia pathogen in the fourth week of the disease (in both cases the agglutination titer of the serum increased from 1:40 to 1:640). Convalescence was slow, and the normal feeling of well-being was regained only after several months.

Subsequently, in the laboratory during the performance of experiments with the spraying of large doses of bacteria a gas mask was used instead of a cotton-gauze mask and this protected against the infection.

What has been stated shows that in those people who recovered from tularemia the immunity developed, being preserved many years, protected very reliably against the development of the disease in contact with the pathogen under ordinary epidemic or laboratory conditions. However, this immunity is not absolute and can be overcome to varying degrees with the entrance of large doses of the pathogen into the organism, particularly through the respiratory tract which is more vulnerable to the infectious disease. Laboratory experiments on guinea pigs, which will be described below, confirm this conclusion. In the Soviet literature recurrences of tularemia, sometimes several years after the initial disease, have been described repeatedly. In these cases, evidently, chronic bacterial carriage occurs as the result of the individual inadequacy of the defense forces of the body. However, in man, just as in animals which possess tularemia sensitivity similar to man (white rats, rabbits, etc.), these cases are only an exception rather than the rule.

#### Immunity from the Injection of Living Tularemia Vaccine

**The Main Properties of the Vaccine Culture.** We consider it necessary to precede the discussion of this problem with a brief review of work which has led to obtaining a living tularemia vaccine. Attempts to use living attenuated cultures for immunization against tularemia were first made by Francis (1929) in a laboratory experiment and

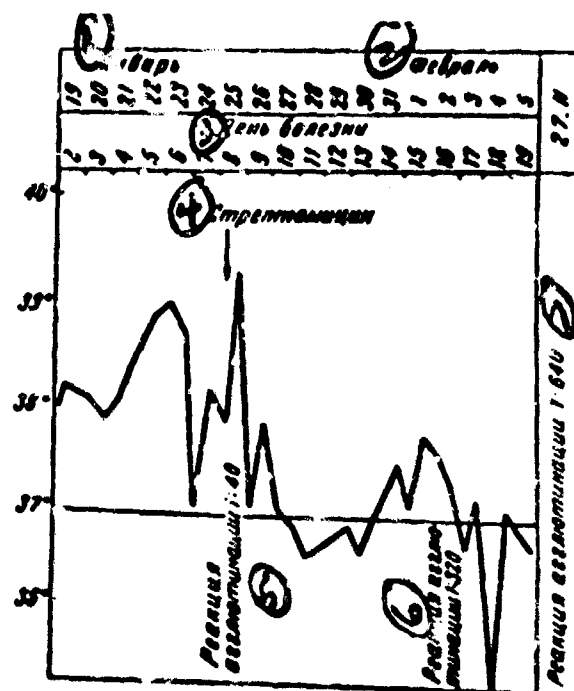


Fig 67. Temperature Curve in a Vaccinated Person Exposed to Accidental Infection with Tularemia by Aspiration. 1. January; 2. February; 3. Day of disease; 4. Streptomycin; 5. Agglutination reaction, 1:40; 6. Agglutination reaction, 1:320; 7. Agglutination reaction, 1:640.

then by Kudo (1934) and Gotschlich and coauthors (1940), but the results of the studies of these authors were inadequate for practical solution of this problem.

Priority in the successful development of a living tularemia vaccine belongs entirely to the Soviet scientists B. Ya. El'bert and N. A. Gayskiy. The authors made a detailed study of the main rules and regulations of tularemia infection and the mechanisms of immunity in it. After being convinced of the practical ineffectiveness of killed vaccines in numerous experiments and considering immunity non-sterile, being realized in accordance with the premunition principle of Sergeant-Donatienne, they concentrated their efforts on developing a living vaccine. In the combined work of B. Ya. El'bert and M. A. Gayskiy published in 1941 report is made of a weakly virulent strain with typical properties of an O (H) culture, the subcutaneous injection of which into guinea pigs and white mice was practically harmless for the animals and was accompanied by the elaboration of an immunity in them of a high degree of strength to subsequent infection with several thousand MLD of a virulent culture. The weakening of the virulence of the strain in the museum occurred spontaneously (quoted from A. L. Matskevich, 1952). The weakly virulent and highly immunogenic strain studied by the authors (which they called "Moskva") was tested on 10 volunteers and was found to be generally harmless when given to them subcutaneously in a dose of 50,000,000 microbes. In those inoculated the allergic reaction was distinctly positive after two-three weeks; the agglutination test was positive in serum dilutions of 1:20-1:160 (quoted from A. L. Matskevich, 1952). This strain was afterwards lost.

N. A. Gayskiy continued these studies and, using methods of directed variation, obtained two attenuated strains in 1941 -- strain 15 ("bouillon", 17th generation) and Muskrat IV ("dry", sixth generation). (In I. N. Mayskiy's work (1953, page 91) it has been pointed out erroneously that the vaccine strain Muskrat IV was obtained from a virulent strain by the same attenuation method as the 15 strain and that the original virulent strain was isolated in 1943). The Muskrat [the Russian name is "Ondatra"] IV strain was attenuated to a greater degree than strain 15. For example, after subcutaneous inoculation of the former strain white mice survived doses of 500,000 and 5,000,000 microbes, and even a dose of 50,000,000 was not the MLCD, whereas 15-20 percent of the mice survived the 15 strain only after the injection of a dose of five microbes; from larger doses all the mice died (N. A. Gayskiy, 1943). Guinea pigs tolerated inoculation with Muskrat IV strain in a dose of up to 500,000,000 microbes; of strain 15, in a dose of 2,500,000 microbes (higher doses were apparently not tested). The author called the first culture a "strain with residual virulence";

the second, a "weakly virulent strain". Pathological studies of guinea pigs vaccinated with Gayskiy's 15 strain made by V. V. Donskov clarified the benign nature of the inflammatory changes found in the regional lymph nodes and partially in the internal organs of the animals. This problem will be presented in greater detail below.

With the aim of clarifying the degree of stability of the properties of the strains obtained Gayskiy passaged the Muskrat IV strain five times through white mice. The passaged culture continued to be avirulent for guinea pigs, but for white mice its virulence was increased -- from a dose of 5,000 microbes or more the mice died.

Strains 15 and Muskrat IV were tested by the author on 50 volunteers under the general clinical observation of V. V. Kosmachevskiy. Considering the greater residual virulence of strain 15 it was given subcutaneously in a dose of only 5,000 microbes, whereas strain Muskrat IV was given in doses of 50,000 and 500,000. Without going into the details of this test, which in general proceeded quite satisfactorily and has been described in detail by V. V. Kosmachevskiy (1944) (see Chapter X), we should like to point out only that it, after showing the practical harmlessness of vaccine cultures obtained by N. A. Gayskiy and their immunological effectiveness, emphasized the need for further improvement of these cultures and supplementary work-out of vaccine doses which would be optimum for injections in people.

This problem was solved successfully by N. A. Gayskiy in his subsequent studies. Using the attenuation method which had been developed, Gayskiy by 1944 had obtained several attenuated cultures with similar degrees of residual virulence for mice and, by the same token, showed that they had not been obtained by chance but rather according to rule. However, of all the vaccine cultures which Gayskiy obtained only strain 15 was relatively stable when subsequently kept in a museum, whereas all the other cultures, including the Muskrat IV strain, lost their valuable properties in the next few years.

I. N. Mayskiy (1949) and then O. S. Yemel'yanova (1949), using N. A. Gayskiy's methods as well as some others, were able to attenuate a number of virulent tularemia strains and obtain vaccine O (Vi) cultures from them. Mayskiy noted that cultures with a very slight residual virulence for mice can be immunogenic, although they are unsuitable for vaccination of people.

Any virulent strain submitted to attenuation but with different degrees of intensity depending on the method used; seven-10 months were adequate for attenuating even the most "resistant" strain. It was made clear through the further studies of O. S. Yemel'yanova that the greatest difficulties do not lie in the attenuation but rather in the stabilization of the properties of the vaccine culture obtained, which is in

agreement with statements made by Gayskiy. These problems are analyzed in greater detail in Chapter III.

American investigators Downs and Woodward (1949), Eigelsbach and others (1951) reported on the possibility of effective immunization of white mice with living cultures (Jap, LR and other strains) attenuated as the result of prolonged keeping on synthetic nutrient media. The residual virulence of the Jap strain (with which the majority of experimental studies published were made) apparently is greater than that of the vaccine strains used in the USSR.

The investigations of B. Ya. El'bert and co-workers clarified the possibility of using the vaccine percutaneously, which very much simplified its incorporation into practice and improved the quality of vaccination. We shall discuss this matter in greater detail somewhat later. Here, we should like to note only that the ability of the vaccine culture to penetrate into the body through the scarified skin and assure the production of immunity of adequate strength is undoubtedly associated with the invasive capacity and residual virulence of the culture. The development of effective methods of drying the vaccine by M. M. Faybich and co-workers assured the possibility of making a high quality preparation which could tolerate prolonged standing.

We should like to dwell on the main properties of tularemia vaccine strains in the form in which this is represented at the current level of our knowledge. The main characteristics of the vaccine strains were given in the works of N. A. Gayskiy. He believed that vaccine strains should possess residual virulence for white mice, that is, they should in doses of no more than 1,000,000 microbes produce death of 30 percent of the white mice used in the experiment after subcutaneous injection but should be harmless in doses of 1,000,000,000 microbes for guinea pigs; the strains should be good allergens and should be agglutinated by specific serum. Such strains (with the use of definite doses injected) protect 90-100 percent of the animals from death when infected with 1,000 lethal doses of a virulent culture. For the human body the vaccine strains should be tolerated in doses of 50,000,000 microbes after subcutaneous injection; these doses should be adequate for the production of immunity which can be demonstrated by the skin allergic test. To what has been stated it may be added that the immunogenicity of the vaccine strains is to a certain degree correlated with their residual virulence; this, evidently, is brought about by the fact that both properties are associated with the presence of the Vi antigenic complex in the cells. The attenuation of the residual virulence of a vaccine strain constitutes the first signal of reduction in its immunogenicity. Strains with residual virulence of less than 30 percent for mice are inadequate in an immunogenic respect.

According to existing instructions for the preparation and testing of tularemia vaccine, complete vaccine strains possess the following properties (O. S. Yemel'yanova, 1957). They are small gram-negative cocci, somewhat larger than the bacteria of virulent strains which possess mucus, are pale-staining by all the usual stains. The vaccine culture agglutinates well with specific serum with the formation of a stable agglutinate which breaks down into large clumps on shaking (Vi agglutination). The number of non-immunogenic cells in the population should not exceed 20 percent; the residual virulence for white mice should be no less than 30 percent and no more than 50 percent (a residual virulence of 50 percent can be attained only in occasional experiments, which is associated with variations in the sensitivity of white mice by seasons, feeding conditions, etc.), which means an average mortality rate of the mice after the subcutaneous injection of doses from 100 to 1,000,000 microbes according to the GKI bacterial standard. No less than 90 percent of the surviving mice are resistant to subcutaneous infection with 1,000 lethal doses of a highly virulent strain. The vaccine strains in a dose of 1,000,000,000 cells are harmless to guinea pigs weighing no less than 400 grams after subcutaneous injection. The strains produce a local inoculation reaction expressed in hyperemia and infiltration of the tissue no less than 0.5 centimeter in diameter after percutaneous (by means of scarification) administration of a suspension containing 1,000,000 microbes according to the GKI standard per cc to guinea pigs.

In the standard method of percutaneous application two drops of the vaccine suspension are applied to the skin of the guinea pig at a distance of two-three centimeters from each other, and then two parallel scratches are made through each drop with a vaccination quill (until blood appears); these scratches are each one centimeter long. Then, the suspension is carefully rubbed in for a minute. The skin inoculation reaction appears no later than two-three days after the inoculation.

In the case of testing on people a suspension of dried vaccine culture containing (after dilution) 1,000,000,000 microbes per cc (according to the GKI standard) should be successful after percutaneous application in no less than 24 out of 25 persons inoculated. The vaccine process should have a benign course, being accompanied by a moderate local skin reaction with the absence, as a rule, of a general reaction. After 30 days, the inoculees should have a positive allergic test with tularin and an agglutination reaction with a serum dilution of 1:20 or higher. At the present time, a vaccine culture which is dried in ampules under vacuum conditions is used for the vaccination of people. Such a vaccine preserves its properties completely for a

year when standing at a temperature of  $+4$  to  $+10^{\circ}$ . Mass growth of the culture for vaccine production is accomplished either on solid nutrient media or on liquid media; in the latter case, this is done with the use of the subsurface method (aeration), which is most productive (L. S. Kolyaditskaya). An important factor in vaccine production is the elimination or reduction to a minimum of phenomena of dissociation in the multiplying bacterial population (see Chapter III). Streaking petri dishes with Yemel'yanova's medium, which makes it possible to determine the number of living bacteria in the preparation and the number of them which are immunogenic, serves as a simple and fully accurate method of checking on the quality of the vaccine prepared. The vaccine being produced should contain no less than 1,000,000,000 living bacteria per cc (after dilution in a solvent); in this number, no less than 50 percent should be immunogenic O (Vi) cells.

In our subsequent presentation we shall deal with the data of study of the Gayskiy 15 vaccine strain or other vaccine cultures which are similar to it in their main properties. In our laboratory a number of investigations was made also with a culture isolated from the dry NIEG [Scientific Research Institute of Epidemiology and Hygiene] vaccine prepared from two strains: Gayskiy 15 and Faybich and Tamarina 10. Judging by the studies of I. N. Mayskiy (1953), strain 10 is somewhat inferior to strain 15 with regard to residual virulence in experiments on mice, but with respect to immunogenicity they are similar. The Gayskiy 15 strain was used for many years in the USSR as the main one in vaccine production, and only in recent years, because of a partial attenuation of it, has it been replaced by a reconstituted 15 variant and by a new strain, 155 (O. S. Yemel'yanova, 1957).

**Subcutaneous, Percutaneous and Intradermal Vaccination.**  
**Immunity from Subcutaneous Injection.** As is well known, the percutaneous method of vaccination of people against tularemia is used in practice, but we consider it necessary to begin with a presentation of studies made with the use of subcutaneous injection of the vaccine culture. By means of this method, which made it possible to dosage the vaccine accurately, the main studies were made on animals and the dose tests were made on people (N. A. Gayskiy, 1943, 1944, 1948; M. M. Faybich and T. S. Tamarina, and others).

The invasive and immunogenic capacities of the vaccine culture, shown in its immunizatory effect after a minimum injected dose, finds its reflection in the species characteristic of the macro-organism, which determine the degree of its susceptibility and immunizability to this culture. According to the data of O. S. Yemel'yanova, white rats are most easily immunized by the vaccine (Table 25). For examples, doses of one and 10 microbes of the restored 15 vaccine



strain immunized all animals used in the experiment, preventing their deaths from subsequent infection with a certain lethal dose of 1,000,000,000 microbes of a virulent strain. The high degree of immunizability of white rats to tularemia antigen is also confirmed in experiments with killed or chemical vaccines. According to the data of the same author, white mice have to be injected subcutaneously with one microbe of a vaccine culture and guinea pigs with 1,000 microbes in order to produce immunity assuring no less than 90 percent survival of the animals with subsequent infection with 1,000 lethal doses of the virulent culture.

Table 25

Immunogenicity of the Reconstituted 15 Strain as a Function of the Dose Used for Subcutaneous Inoculation and the Species of Animal (after O. S. Yemel'yanova)

1 Доза культуры (миллионные клетки)	2 Белые мыши						3 Морские свинки				4 Белые крысы			
	иммунизация			результаты контрольного заражения			результаты контрольного заражения		результаты контрольного заражения		результаты контрольного заражения		результаты контрольного заражения	
	7 пало всего	8 пало при иммунизации (остаточная вирулентность)	9 %	10 заражено	11 пало от туляремии	12 выжило	13 %	14 иммунизировано	15 пало от туляремии	16 выжило (оказалось иммунным)	17 %	18 иммунизировано	19 пало от туляремии	20 выжило (оказалось иммунным)
1	50	1	2	49	3	46	94	19	12	7	10	0	10	10
10	68	8	12	60	1	59	98	20	3	17	10	0	10	10
100	137	29	21	108	2	106	98	19	3	16	10	0	10	10
1 000	137	47	34	90	1	88	99	20	0	20	10	0	10	10
10 000	137	59	42	78	0	78	100	18	1	17	10	0	10	10

Note. Immunity in the white mice was checked 20-25 days after vaccination by means of a control subcutaneous infection with a dose of 1,000 microbes; in guinea pigs, after 30 days with a dose of 1,000 microbes; in white rats, after 30 days with a dose of 1,000,000,000 microbes of a virulent tularemia strain. Simultaneously, non-immune white mice and guinea pigs infected subcutaneously with a dose of one and 10 microbes of the same virulent strain, and white rats infected with a dose of 1,000,000,000 microbes died of tularemia. The animals which died from other causes during immunization or control infection are not included in the Table. 1. Dose of culture (microbes); 2. White mice; 3. Guinea pigs; 4. White rats; 5. Immunized; 6. Results of control infection; 7. Total; 8. Died during immunization (residual virulence); 9. Infected; 10. Died of tularemia; 11. Survived; 12. Survived (were found to be immune).

According to the data of M. M. Faybich and T. S. Tamarina (1946), 90 percent survival after a control infection with a virulent culture was observed only in the event that the white mice had been immunized with a dose of 100 microbes of the vaccine strain and guinea pigs, with a dose of 1,000 microbes. In the experiments of N. A. Gayskiy (1944) the strains which he had attenuated in the smallest doses (one; two or five microbes) protected all mice and guinea pigs against death from subsequent injections of large doses of the virulent culture. This can serve as an indication of the fact that in Gayskiy's experiments the vaccine strains (including strain 15) were in a less attenuated state than in the experiments of Faybich and Tamarina.

Doses of 500,000,000 and 1,000,000,000 microbes of a vaccine culture are usually not lethal to guinea pigs, but a dose of 3,000,000,000 is lethal (M. M. Faybich and T. S. Tamarina, 1946). In contrast to guinea pigs and white rats, white mice show a certain degree of infectious sensitivity to the vaccine culture which is expressed in the death of part of the animals even from relatively small doses. This afforded N. A. Gayskiy the basis for considering vaccine tularemia strains as cultures which contain residual virulence. The number of deaths in the white mice after the injection of a vaccine strain depends on the dose of it. According to the data of O. S. Yemel'yanova five-20 percent of the mice die, respectively, from doses of one-100 microbes on subcutaneous injection; 33-58 percent, from 1,000-1,000,000 microbes; 81-100 percent of the mice, from 100,000,000 to 1,000,000,000 microbes (Table 26).

According to the data of the same author, the survival time of white mice from vaccinal infection, in contrast to the short survival periods of mice after infection with highly virulent strains, can drag on up to 19 days, although the average survival time is within the limits of five-10 days. Death of the mice most often occurs on the sixth-ninth day after doses of one-1,000,000 microbes; by the 10th day 80-90 percent of the mice die, after which death of isolated animals is observed. In contrast to what occurs after acute infection in mice, when the microbes, as a rule, are found in large numbers in smears taken from the spleen, in the case of death of mice from the vaccine strain bacterioscopy of the spleen is far from always being positive, and the degree of seeding of it is considerably less than after infection with a virulent culture; frequently, in many fields it is possible to detect only isolated microbes (a slightly positive or doubtful bacterioscopic result). The bacterioscopic results are directly related to the survival time of the mice and only indirectly to the dose of infection. In cases where death occurs early (up to the sixth day inclusive) reliable bacterioscopy is observed in almost all the mice which die; in those

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Table 26

Death of Mice from Doses of One-1,000,000,000 Microbes of Different Vaccine Strains (after O.S. Yemel'yanova)

1 Доза (микро- бы миллион)	2 Число вакци- нированных мышей	3 Пало от вакциновой туляремии		5 Сроки гибели (сутки)	
		4 число	%	6 среднее	10 от-до
1	575	28	5	10.2	6-19
10	374	44	12	10.2	7-14
100	561	112	20	9.6	5-19
1 000	645	213	33	8.9	5-18
10 000	549	184	34	8.6	5-17
100 000	321	153	47	7.8	4-17
1 млн. 7	549	318	58	7.1	3-19
100 " 8	210	171	81	5.0	2-14
1 млрд.	50	50	100	3.0	2-4
Всего 9	3834	1273	33	7.8	2-19

Note. Data on vaccine strains the properties of which satisfy the requirements of the instructions are included in the Table. 1. Dose (microbes); 2. Number of mice vaccinated; 3. Died of vaccine tularemia; 4. Number; 5. Survival time (days); 6. Average; 7. Million; 8. Billion; 9. Total; 10. From-to.

which die on the seventh-10th day, in only 17-66 percent; later, from zero to six percent of the cases. Microbes are found extremely rarely in the blood.

The pathological picture in mice which die of vaccine tularemia in periods within the eighth-10th day, when the microbes can no longer be found bacterioscopically in a number of cases, is characterized by the following: at the site of injection there is a dense infiltrate; the regional lymph nodes are enlarged, hyperemic; the spleen and liver are of increased density and enlarged; in the case of death in the later periods no characteristic pathological changes are found macroscopically. The most reliable method of proving death of mice from vaccine tularemia is constituted by cultures of spleen on coagulated yolk medium, which inevitably (in the absence of extraneous flora) gives a positive result even from animals which die in the remote period.

However, in these cases the growth of bacteria may be sparse and will appear only after the cultures are kept for a long time (up to 10 days) in the incubator (O. S. Yemel'yanova).

Rabbits need to be injected with quite considerable doses of the tularemia culture vaccine in order to obtain an immunizing effect. In the experiments of N. A. Gayskiy (1943), after subcutaneous injection of rabbits with a dose of 5,000,000 microbes of the 15 strain allergic reorganization was noted in only part of the animals, while after the administration of a dose of 50,000,000 microbes it was found in all animals. According to the data of O. S. Yemel'yanova, subcutaneous injection of rabbits with a dose of 100,000 microbes of the 15-reconstituted strain caused the formation of agglutinins in all five animals used in the experiment, but the titers were low, not exceeding 1:20. After the injection of a dose of 10,000 microbes the appearance of agglutinins was noted in only two out of five rabbits, and after the injection of a dose of 1,000 microbes antibodies were not found in the same number of experimental animals even with a serum dilution of 1:5. In the case of subcutaneous inoculation of rabbits with a dose of 1,000,000 microbes or higher agglutinins were then found in all the experimental animals, and the agglutination titers (by the 20th day) were the higher the greater the dose of vaccine. One month after vaccination the rabbits were infected subcutaneously with 10,000,000,000 microbes of the fully virulent 503 strain. All the rabbits vaccinated with a dose of 1,000,000,000 microbes or more as well as one out of five rabbits immunized with a dose of 100,000 microbes survived. The remaining rabbits, like the 10 controls, died of acute tularemia after three-four days.

The human body is characterized by a moderate susceptibility to the vaccine culture -- quite considerable doses are necessary to surmount the natural defense forces of the body and produce immune reactions. For adults doses of 10,000,000 to 25,000,000 microbes are optimal for immunization with subcutaneous injection; a dose of 1,000,000 or 5,000,000 microbes is inadequate (N. A. Gayskiy, M. M. Faybich and others). This problem will be discussed in greater detail in Chapter X. As far as the infectious sensitivity of the human body is concerned, in this case it is negligible, since the vaccine culture is practically harmless for man.

#### Immunity after Percutaneous and Intradermal Injections.

B. Ya. El'bert (1945) proposed the percutaneous method of vaccination against tularemia, first checking it in experiments on guinea pigs. Rubbing the culture of vaccine into a linear superficial scratch on the shaved guinea pig skin produced a vaccine process in them, and after a month the animals were resistant to subcutaneous and other methods

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of infecting them with a virulent culture of tularemia bacteria. Later, in studies published by this author and his co-workers (B. Ya. El'bert and coauthors, 1947) the results of subsequent experimental investigations are reported, which made it possible to characterize completely the effectiveness of this vaccination method. It was determined that in experimental guinea pigs the inflammatory changes in the skin at the site of application of the vaccine as well as in the regional lymph nodes are of a benign character. Percutaneous vaccination was accompanied by antibody production (agglutination titers of the sera were 1:25-1:50) and a specific allergic reorganization of the body as demonstrated by the intradermal tularin test. The guinea pigs vaccinated percutaneously show resistance to percutaneous, subcutaneous, conjunctival, intranasal, alimentary, and air-droplet infection with a virulent culture, whereas all the control unvaccinated animals die.

Laboratory data obtained made it possible for B. Ya. El'bert and co-workers to proceed with percutaneous vaccination of people. The inoculations were harmless and were well tolerated. In the inoculees agglutinins were then found in serum dilutions of 1:200-1:400, and the skin allergic reactivity was found to the injection of tularin (for more details see Chapter X).

In proposing the percutaneous method of vaccination against tularemia B. Ya. El'bert (1946) pointed to the following advantages of it: 1) gradual development of the vaccine process in which the microbe of the tularemia vaccination first multiplies in the skin and then invades the deeper cellular systems and, finally, reaches the regional lymph nodes, that is, percutaneous immunization is expressed in a stage infiltration of the microbes from the skin into the depth of the tissues in fractional doses from the skin depot formed; 2) percutaneous vaccination permits a direct reading of the success of the inoculation, based on the formation of a typical succession of reaction phases on the skin of those vaccinated along the course of the scarified areas; 3) percutaneous vaccination consists of the use of a simple technical method in the form of rubbing the vaccine into a linear scratch in the skin, as in the case of a smallpox vaccination, which fully assures the possibility of mass coverage of people in localities which are epidemiologically unfavorable for tularemia.

N. A. Gayskiy and co-workers (1949), after checking experimentally and then checking on people the percutaneous method of vaccination against tularemia proposed by B. Ya. El'bert, praised it highly. Like B. Ya. El'bert, he pointed to the important role of the skin in the development of immunity in tularemia and he indicated the theoretical basis of utilization of the skin as an "organ of immunogenesis".

As is well known, the method of vaccination through the skin has been immunologically very effective in a number of infectious diseases, particularly smallpox, tuberculosis and plague. The percutaneous method of vaccination in tularemia has fully justified itself in antiepidemic practice, and at the present time this method is used exclusively (see Chapter X).

In the experiments of O. S. Yemel'yanova a dilution of  $10^{-3}$  of a one-billion vaccine culture suspension (that is, 1,000,000 microbes are present in one cc) was adequate to assure resistance to subsequent infection with 1,000 MLCD of the virulent 503 strain after percutaneous application to guinea pigs in all the vaccinated animals. A dilution of  $10^{-4}$  created an immunity of adequate strength in the majority of guinea pigs, whereas subsequent dilutions were practically ineffective. Almost the same results were obtained by V. A. Yudenich (1954) in the testing of a freshly-prepared liquid egg yolk vaccine which had 1,000,000,000 living bacteria per cc. The rabbits could be vaccinated percutaneously by using a one-billion suspension of the vaccine culture (O. S. Yemel'yanova).

The number of bacteria introduced into scratches on percutaneous vaccination has not been determined by anyone. Undoubtedly, it is subject to variations depending on the number and the length of the scratches made, their depth, the intensity with which the vaccine is subsequently rubbed in, etc. (V. G. Pilipenko and T. A. Shchekina, 1959). Without violating the principle of vaccination through the skin, with the aim of a more accurate control of the dosage the intradermal method of injecting the vaccine can be used in the laboratory experiment. In our laboratory this method is used frequently for the immunization of guinea pigs in those cases where it is necessary to know the dose of vaccine accurately. The dose of 10,000 microbes injected intradermally usually immunizes all or almost all of the guinea pigs used in the experiment, but for the purpose of obtaining a more complete effect of vaccination it is necessary to inject the animals with larger doses, for example, 10,000,000 microbes (T. A. Kalitina). In the experiments of O. S. Yemel'yanova on percutaneous vaccination of guinea pigs and rabbits by means of the standard method of application of the vaccine, it was noted that with the use of a suspension with a concentration of 1,000,000,000 microbes per cc the immunological effect of vaccination, determined on the 20th and 30th days by the level of the agglutination titer of the serum, was less than after subcutaneous injection of the same dose, but a little higher than after subcutaneous injection of a dose of 100,000,000 microbes.

In people, the dry vaccine even in a tenfold dilution ( $10^{-1}$ ) usually produces a very distinct inoculation reaction in 60-100 percent

of the cases depending on the series of vaccine used (L. S. Matveyets, G. P. Uglovoy and others). These data show that in the vaccine being produced several minimum immunizing doses are present in a single dose, which is very important for insuring the quality of the vaccine during the time it stands, when a partial extinction of the microbes occurs.

In very thorough experiments of V. P. Motornaya (1953) in guinea pigs it was made clear that subcutaneous, intradermal and percutaneous methods of vaccination are practically equivalent with regard to the immunizing effect.

Morphology (Histopathology) of the Vaccine Process Experimentally. Histological studies of the organs of animals vaccinated with Gayskiy vaccine culture were made for the first time by V. V. Dunskov (1944). The author had at his disposal the organs of guinea pigs, which were subcutaneously inoculated with doses from 1,000 to 5,000 microbes of the 10th-17th generations of the 15 strain. According to Dunskov's data the most distinct changes were found in the lymph nodes, regional to the site of injection of the culture, and were expressed in the formation of typical tularemia granulomas in their tissues which subsequently underwent fibrosis for the most part. In part of the cases the granulomas underwent necrotization, and in three guinea pigs out of 30 a secondary suppuration of the necrotic foci was found. Specific granulomas were found in the spleens of eight out of 30 guinea pigs, but in these cases the inflammatory process was of a productive character, and in only two guinea pigs were there small foci of necrosis in the centers of the granulomas. In the liver no specific inflammatory changes were found, but in the lungs, in part of the guinea pigs, moderate changes were noted in the form of hyperemia, edema, and cellular infiltration along the courses of the bronchi and blood vessels. The author notes that the later generations of the 15 strain produced correspondingly less pronounced pathological changes in the guinea pigs, which indicated a gradual reduction in the residual virulence of the strain.

I. A. Chalisov and M. G. Spasskaya (1946, 1948), and then Z. D. Khakhina (1947), investigating the organs of guinea pigs which had been vaccinated subcutaneously or percutaneously, and A. V. Mashkov (1952), investigating the organs of guinea pigs, white mice and white rats which had been vaccinated, also noted the benign character of the pathological changes in the tissues from the vaccine process. In the generalized form the results of the studies of the authors mentioned are the following. After subcutaneous and percutaneous injection in guinea pigs and subcutaneous injection of mice and white rats using immunizing doses of the vaccine culture the latter

regularly penetrates into the internal organs and produces a specific inflammatory granulomatous process in the tissues. In white mice, as animals more sensitive to tularemia, this process is more pronounced and is accompanied by partial destruction of the tissue, whereas in guinea pigs it has a less diffuse character, and destructive changes in the granulomas are rare. Finally, in white rats, even after the injection of a very large dose of vaccine, the inflammatory changes are perfectly benign, and they quite rapidly undergo resolution, which indicates the rapid production of immunity (A. V. Mashkov). I. A. Chalisov and M. G. Spasskaya (1946) noted the duration of the vaccine inflammatory process in guinea pigs, tentatively defining it as no less than three months. The state of allergy in guinea pigs occurring during the process of vaccination is confirmed well, in the authors' opinion, by characteristic changes in the blood vessel walls in the focus of inflammation (at the site of injection of the culture) and the lymph nodes during the initial, acute period. After inoculation with the Gay-skiy vaccine strain V. V. Donskov (1944) did not find any macroscopic or microscopic changes in rabbits. Evidently, this is associated with the inadequate dose of the vaccine -- the author points out that the animals were injected with 1,000 to 5,000 microbes.

A. V. Mashkov (1952) indicates that during the entire observation period (43 days) he found bacteria phagocytized by mononuclear cells by microscopy of the spleen and liver, whereby the picture of phagocytosis was of the same character as in the experiments with the virulent culture. In view of the fact that these observations were not reinforced by bacteriological studies and were made without use of selective staining of the bacteria they need further confirmation.

**Bacteriology of the Vaccine Process Experimentally.**  
Study of the bacteriology of the vaccine process runs into difficulties associated with the inadequate development of methods of detecting attenuated bacteria in the organs and tissues of vaccinated animals. Judging from experiments with a virulent culture (see Chapter IV), bacteria can be found relatively regularly by the method of streaking (with an impression of a piece of the organ) on synthetic nutrient medium only if the number of them per gram of tissue of the investigated organ comes to 1,000,000 or more. The biological method of detection of bacteria, so successful in working with a virulent culture, should be supplemented by the immunological method (that is, by checking the immunity in animals used for the biological test) in experiments with a vaccine culture, which complexifies the analysis. However, the promise of this method is clearly shown in the works of N. A. Gayskiy and then those of T. N. Dunayeva.

After the injection of a vaccine culture the infectious



process in animals susceptible to it passes through those phases of adaptation, regional infection and hematogenous dissemination (focal spread) as after infection with a virulent culture. However, this process, even in the most sensitive animals, occurs in a much more benign manner and on a lower level of seeding of the organs and tissues with bacteria than after infection with a virulent culture. In animals of group I (white mice, voles, lemmings) small doses of the vaccine culture produce a lingering process with prolonged circulation of bacteria in the blood. N. A. Gayskiy (1946, 1948) notes that in white mice which have been vaccinated cultures made from their organs (spleen and bone marrow) after 20 days are positive in 40 percent of the cases. The author succeeded in showing, in a certain percentage of cases, the existence of living microbes in the bodies of the vaccinated mice by immunological methods at even later periods after vaccination (after 36 and 67 days). He emphasizes the need for the penetration of vaccine bacteria into the internal organs of the animal being immunized for the production of a strong immunity.

Longer periods of detection of bacteria (up to four months) were found by T. N. Dunayeva in steppe lemmings [*Lagurus lagurus*], which show increased sensitivity to the vaccine culture by comparison with other animals of group I. In the experiments of T. N. Dunayeva on common voles [sometimes known as the Northern vole† *Microtus arvalis*] it was made clear that even with a small infective dose (100 microbes subcutaneously), which usually produces a benign process in these animals which ends in recovery, the vaccine culture penetrates into the blood stream and is found here from the third-fourth through the 20th day (the observation period). For the purpose of detecting bacteria in the blood the author fed *Dermacentor marginatus* larvae on the voles and studied them according to the days on which they fell off, inoculating white mice with subsequent check of their immunity to subcutaneous injection of 100 MLCD of the virulent strain. After the injection of a dose of 100,000,000 microbes of the vaccine into the voles, which is lethal for them, bacteria were found in the blood as early as after 24 hours and were found regularly in it until the animals died (Fig 68).

The injection of white mice and other animals of group I with relatively small doses of a vaccine culture usually causes moderate injury to the tissues in organs which fix the bacteria but does not paralyze immunity development, and this leads to a localization of the infection and subsequent gradual subsidence of it. While in acute tularemia caused by infection with a virulent culture the granulomatous changes and tissue necrotization are intensified with the development of the infectious process, in experiments with Gayskiy's vaccine culture

†Also known as the meadow or field vole.

(3)  
биопробы на белых мышах из суспензий наросавшихся личинок клещей по дням отпадения их с вакцинированных полевок

Доза вакцины в микробах (1)	Вакцинированные полевки, на которых личинки клещей (2)	1	2	3-4	5	6	7	8	9	10-11	12	13	14	15	16	17-18	19	20	21
100	(1) (2)	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
	(3) (4)	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
100 МЛД (4)	(1) (2)	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
	(3) (4)	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
	(5) (6)	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗

Полевка пала (5)

Fig 68. Time of Finding Vaccine Culture in the Blood of Common Voles by the Method of Feeding Dermacentor Marginatus Larvae on Them with Subsequent Investigation of Them by the Biological Test on White Mice. Each circle corresponds to one animal. Circles with ordinary hatching denote the animals which died of the vaccine infection; circles with cross-hatching show the animals which survived and which were found to be immune to subsequent infection with 100 MLCD of a virulent culture; the unhatched circles represent the surviving animals which were not immune to subsequent infection with 100 MLCD of a virulent culture (according to T. N. Dunayeva). The figures in the circles show the survival time of the animals after vaccination (or infection). 1. Dose of vaccine in microbes; 2. Vaccinated voles on which the tick larvae were fed; 3. Biological test on white mice from suspensions of tick larvae which had been attached to the voles according to days on which they fell off the vaccinated voles; 4. 100,000,000; 5. The vole died.

the predominance of the productive phase of inflammation is noted, the resolution of granulomas, and reparative changes (A. V. Mashkov). With recovery the elimination of vaccine bacteria from the body occurs in animals of the first group slowly, but is still completed by two months in the majority of cases, although in occasional individuals it

can drag on to four months and possibly even longer.

After the inoculation of large doses of vaccine into white mice and other animals of group I the injury to the tissues of the liver, spleen, bone marrow and other organs reaches a considerable degree, and the phase of hematogenous dissemination (and of focal spread) is usually replaced by septicemia which ends in death. However, death occurs with much less seeding of the organs than after infection with a virulent culture (I. S. Tinker and M. S. Drozhevskina, 1948; O. S. Yemel'yanova). This indicates partial resistance of the body, despite the injection of a massive dose of vaccine. In cases of survival of animals of group I after injected with relatively massive doses of the vaccine resolution of the inflammatory changes in the organs occurs more rapidly than after the injection of small doses (A. V. Mashkov, 1952). This can indirectly indicate the more rapid elimination of bacteria from the body as the result of more active antigenic influences.

In guinea pigs which, as has been indicated in Chapter IV, possess a slight but clearly expressed natural resistance to the virulent tularemia culture, the vaccine produces a benign process, by comparison with white mice and other animals in group I, even when injected in relatively large doses. After subcutaneous injection of guinea pigs with a vaccine in a dose of 1,000,000,000 microbes the cultures may be regularly positive from the liver, spleen and lungs until the 10th day; from the regional lymph node and site of inoculation, until the 30th day (V. A. Yudenich, 1954). After the injection of smaller doses of the vaccine the cultures are usually positive from tissue taken from the injection site and the regional lymph node until the 15th-20th day and irregularly, from the spleen until the third-fifth day (V. A. Yudenich, 1954; O. S. Yemel'yanova).

The time needed for ridding the guinea pig organism of the vaccine cells which have penetrated into its internal organs and regional lymph node is possibly longer than can be established by the method of inoculation of egg yolk medium, in view of the inadequate sensitivity of this latter for the detection of relatively small numbers of bacteria in the tissue being investigated. I. N. Mayskiy (1953), two-and-a-half months after vaccination of guinea pigs, could not find tularemia bacteria in their organs and considers this proof of the transition of immunity into a sterile phase.

In experiments on rabbits I. S. Tinker and M. S. Drozhevskina (1948) determined the fact that after subcutaneous injection of animals with a dose of 10,000,000 microbes of a vaccine culture the bacteria were then found by streaking liquid yolk medium at the site of injection and in the regional lymph nodes until the 20th day, but in two cases (out of 10) microbes were plated out of the spleen on the third

and fifth days after inoculation.

**The Rate of Formation, Strength and Duration of Immunity.** Experiments on laboratory animals demonstrate in a perfectly clear-cut manner the relatively rapid formation of immunity to tularemia after subcutaneous, intradermal or percutaneous administration of living tularemia vaccine. The rate of production of immunity depends on the dose of vaccine given and, to a certain degree, on the species of animal. Immunity of great strength is created, protecting the animal against the injection of massive doses of a virulent culture.

N. A. Gayskiy and coauthors (1947) report that immunity in white mice appears during the first few days after vaccination; five days after the subcutaneous inoculation of 100 microbes of a vaccine culture 43 percent of the mice showed resistance to infection with 10,000 minimum lethal doses of a virulent culture; after 10 and 15 days, all 100 percent. In guinea pigs vaccinated subcutaneously with a dose of 1,000,000 microbes resistance to an infection of 100,000 MLD of a virulent culture was noted five days after vaccination in 40 percent of the cases; nine days after, in 57 percent; 22 days after, 87.5 percent; 30 days after, the percentage of immune animals comes close to 100. The appearance of immunological reactions -- allergic reactions and agglutination reactions -- was noted by the authors 10 days after vaccination in half of the guinea pigs checked, and after 15-21 days the allergic reaction was positive in all guinea pigs, whereas the agglutination reaction on the 26th day was positive in eight out of 10 guinea pigs, but the serum titer did not exceed 1:10. In the experiments of B. Ya. El'bert and coauthors (1947) guinea pigs withstood subcutaneous infection with 10,000 lethal doses of a virulent strain as early as the third day after percutaneous vaccination.

Similar results in guinea pig experiments were then obtained by A. V. Mashkov (1952), I. N. Mayskiy (1953), T. A. Kalitina (1953) and V. A. Yudenich (1954). After the subcutaneous injection of a quite massive dose of the vaccine culture of the 15 strain (500,000 microbes) part of the guinea pigs, according to I. N. Mayskiy's data, can withstand subcutaneous infection of 10 MLD of a virulent culture after two days; after four days the guinea pigs are capable of standing infection with 100 MLD; after 10 days, 100,000 MLD; after the 15th day, 10,000,000 MLD. Experiments with percutaneous vaccination gave similar results. In experiments with subcutaneous vaccination as early as the fifth day after the guinea pigs reacted to intradermal injection of tularin; on the sixth and seventh days the intradermal test was positive in almost all animals. The agglutination test on the sixth day after vaccination was positive in dilutions of 1:10-1:20 in half of the guinea pigs studied; on the seventh day, in almost all. In experi-

ments with percutaneous vaccination agglutinins were found in almost all guinea pigs beginning with the seventh day, but at this time only two guinea pigs out of eight reacted to tularin, whereas on the 10th day antibodies and skin reactivity were found in all guinea pigs. In experiments with percutaneous vaccination of guinea pigs V. A. Yudenich (1954) obtained data similar to those of I. N. Mayskiy.

Increase in the dose of vaccine makes it possible to bring about the survival of guinea pigs and white mice after an even smaller interval between vaccination and infection than two days. The same result may be obtained from simultaneous injection of the animals with a massive dose of a vaccine culture and several lethal doses of a virulent culture (A. V. Mashkov, 1952; I. N. Mayskiy, 1953). This experiment can be done comparatively easily if the animal is injected with a mixture of both cultures into the same extremity, and is made difficult to some degree if the cultures are injected separately into different extremities. N. N. Ginsburg (1947), based on the results of experiments on rabbits with anthrax and on guinea pigs with plague and tularemia, called this phenomenon a "survival phenomenon", whereby he pointed out that its mechanism is not clear. As is justifiably noted by I. N. Mayskiy (1953), the "survival phenomenon" of Ginsburg represents only a specific case of the "law of reinoculation" of Sh. D. Moshkovskiy. I. N. Mayskiy and A. V. Mashkov conclude correctly that with the combined injection of a massive dose of a vaccine culture and a small dose of a virulent culture the former rapidly reaches a high concentration in the organs and causes immunity, which limits the multiplication of the virulent microbe and therefore prevents the development of an acute tularemia infection.

In the experiments presented on clarification of the rate of development of immunity in vaccinated mice and guinea pigs to infection with doses of a virulent culture known to be lethal it would be incorrect to think that in cases of survival (recovery) of the experimental animals after infection a short time after infection they possess immunity as early as on the day of infection. This is particularly clear with respect to animals which were infected with a virulent culture simultaneously with injection of the vaccine. Evidently, these animals at the time of infection were not immune, although they survived. One of the indices indicating attainment of resistance after vaccination may be the appearance of the first signs of reparative changes in the tissues in the foci of inflammation (A. V. Mashkov, 1952).

Vaccinated white mice and guinea pigs, during the period of maximum development of the immunity, are resistant to infection with massive doses of a virulent culture numbering hundreds of thousands and millions of MLCD (N. A. Gayskiy, B. Ya. El'bert, I. N.

Mayskiy, V. A. Yudenich, and others). In the experiments of O. S. Yemel'yanova white mice survived doses of 100,000-1,000,000 MLCD when infected with a virulent tularemia culture three weeks after vaccination as well, partially, as doses of 10,000,000-100,000,000 MLCD; only after a dose of 1,000,000,000 MLCD do almost all the mice die of tularemia (Fig 69). The result obtained very much resembled that from infection of non-immune white rats with similar doses. In other words, vaccination of the mice lowered their sensitivity to tularemia to the level of sensitivity of animals in group II. A similar statement has been made in the works of A. D. Mashkov (1952).

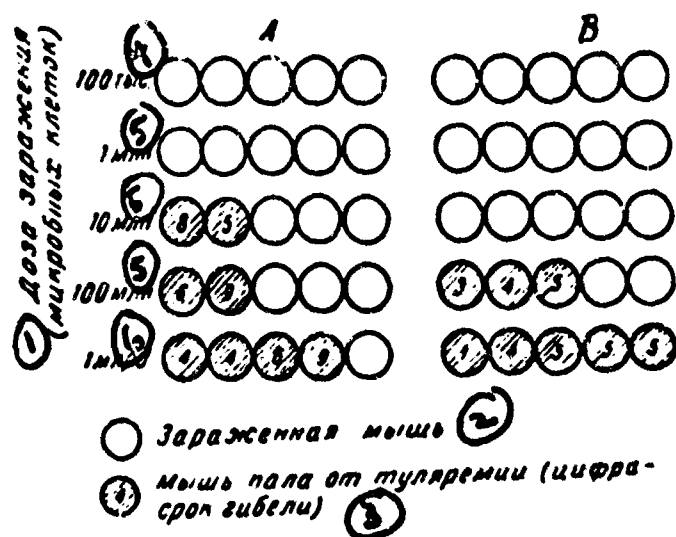


Fig 69. Results of Subcutaneous Infection of White Mice with Different Doses of a Virulent Tularemia Culture Three Weeks after Subcutaneous Vaccination with a Dose of 1,000 Microbes of the NIEG Vaccine Culture. A. Infection with the 503 strain; B. Infection with the 9 strain (after O. S. Yemel'yanova). 1. Infective dose (microbes); 2. Infected mouse; 3. Mice died of tularemia (the figures indicate the survival time); 4. Thousands; 5. Million(s); 6. Billion.

Important indices of the strength of immunity in vaccination are the following: the ability of the body of the vaccinated animal to suppress the multiplication of virulent bacteria which has penetrated into the organism, the nature of inflammatory reactions in the tissues into which the microbes have penetrated, and the rate of elimination of these from the body. I. S. Tinker and M. S. Drozhovkina (1949) point out that in vaccinated white mice, guinea pigs and rabbits virulent tularemia bacteria injected subcutaneously do not spread beyond the

limits of the primary complex -- the site of injection of the pathogen and the regional lymph nodes. However, in the experiments of other investigators it was determined that in guinea pigs immunized with living vaccine the immunity is inadequate to prevent penetration of part of the bacteria into the blood and internal organs after infection with a virulent culture (V. A. Yudenich, 1956; L. S. Matveyets). Similar data are available with respect to white mice (T. N. Dunayeva). Vaccinated guinea pigs, surviving after infection with many lethal doses of a virulent strain, usually have quite pronounced clinical manifestations of disease (fever, loss of weight, enlargement of regional lymph nodes), which clearly indicates generalization of the infection, at least in brief. In animals, after infection, there is a marked intensification of immunological reactions (allergy, agglutination), and specifically the serum agglutination titers increase by three-five times (T. A. Kalitina, 1953; L. S. Matveyets, 1960). Even a triple vaccination (at monthly intervals) with a vaccine culture in guinea pigs does not create immunity in them adequate to limit the virulent infection to the regional phase (L. S. Matveyets). Such immunity can be created in guinea pigs only after acting on them additionally with a virulent culture (see below).

What has been stated cannot be applied to the human body, because in it, on account of the presence of a quite considerable natural resistance to tularemia, vaccination leads to the production of immunity which is many times greater than that of guinea pigs or white mice.

Vaccinated guinea pigs or white mice, after being infected with a virulent culture, sometimes remain bacterial carriers for a long time. For example, in the regional lymph nodes of various guinea pigs it was possible to find virulent tularemia bacteria eight months (the observation period) after infection (R. A. Savel'yeva), whereas in white mice they are found up to 100 days (N. A. Gayskiy, 1948). Even longer periods of preservation of a virulent tularemia bacteria in the bodies of occasional vaccinated white mice (up to 11-1/2 months) have been reported by N. D. Altareva and Ye. P. Potapova (1957). With the aim of detecting the pathogen in the mice the authors resorted to provocation by the subcutaneous injection of 10 percent ethyl alcohol into the animals. Unfortunately, the authors did not mention the activity of the vaccine which they used for creating immunity in the mice or which doses of it were used. In the experiments of T. N. Dunayeva white mice immunized subcutaneously with a vaccine NIEG culture and infected after a month (also subcutaneously) with doses of 1,000 and 100,000 microbes of a virulent strain, were free of virulent bacteria 25-35 days after infection. After injection of a dose of 100

microbes the mice were rid of the pathogen by the 15th-20th day, which was evidenced by the negative results of biological tests of investigation of mouse organs.

The duration of immunity after injecting living vaccine differs in different species of animals. In white mice the immunity was preserved three months, but as early as after four months the majority of animals dies after a test infection with a virulent culture (N. A. Gayskiy and coauthors, 1947). In guinea pigs immunity after vaccination is preserved longer than in mice. N. A. Gayskiy and co-workers (1947) define the duration of immunity in guinea pigs as averaging 10 months, whereas by the 15th month immunity is preserved in only 60 percent of the animals. According to the data of I. S. Tinker and T. I. Puchkova (1948), I. N. Mayskiy (1953) and V. A. Yudenich (1954), vaccinated guinea pigs remain immune (to infection with a virulent culture) for a year, and in part of the cases even longer. In the experiments of A. L. Matskevich (1952), four-seven months after vaccination only 50 percent of the animals were immune, and in the experiments of T. A. Kalitina, L. S. Matveyets and K. N. Shlygina five-six months after vaccination 60-70 percent of the animals were immune. The differences in the time of immunity in the guinea pigs in experiments of the first and second groups of these authors apparently should be related to the difference in the virulence of the strains used for test infection of the animals.

Strength and duration of immunity in laboratory animals infected with a virulent culture after preceding vaccination are much greater than in animals injected with the vaccine alone. White mice subjected to this double influence withstand the infection with a dose of 1,000,000,000 microbes of a virulent culture at the height of development of immunity, and after 20-30 days in the majority of cases it is no longer possible to find tularemia bacteria in their organs. This can serve as an indication of the fact that the higher the level of the immunity attained the more vigorously this sterilizing effect is manifested. Experiments on guinea pigs also show that after the effect of a vaccine and then of a virulent culture on the bodies of these animals immunity of much greater strength is produced in them than after the effect of a vaccine culture alone. Guinea pigs subjected to this double influence preserve their immunity completely after a year, and after infection with 100 MLCD of a virulent strain they practically do not become sick with tularemia (T. A. Kalitina). After intradermal injection of a dose of 10,000,000 MLCD into such highly immune guinea pigs a local skin reaction develops in them similar to the Koch phenomenon, without a general temperature reaction, and inflammatory changes are also noted in the regional lymph nodes. With a small infective dose (10 MLCD)



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the pathogen, as a rule, is not found in the bodies of immune guinea pigs further than the regional lymph nodes; with a large dose (10,000,000 MLCD) it partially penetrates into the parenchymatous organs but is found there in very small quantities (R. A. Savel'yeva). Histological study of the organs of these guinea pigs showed specific inflammatory changes in the regional lymph nodes which were more markedly expressed in animals infected with a large dose, whereas the tissues of the internal organs -- spleen, liver, etc. -- were normal as a rule (A. P. Gindin). These data demonstrate the high degree of protection of the immune organism to reinfection by the parenteral route. In people living tularemia vaccine produces a quite rapid formation of immunity. The practice of work of our laboratory shows that the inoculees (percutaneously) can be permitted to work freely with infectious material two weeks after vaccination if there is a skin allergic reaction to the injection of the usual dose of tularin. These observations include more than 20 inoculees. According to observations in foci of tularemia the appearance of new cases of tularemia stops no later than 10-12 days after percutaneous vaccination (B. Ya. El'bert and coauthors, 1947; V. S. Sil'chenko, 1953), which indicates the time of production of immunity of adequate strength. This problem will be analyzed in greater detail in Chapter X.

After vaccination of people immunity of greater strength is created and it is preserved for a longer time than in guinea pigs or white mice, which should be related to the more perfect protective mechanisms of the human body against tularemia by comparison with those of the animals mentioned above, which possess too great a sensitivity to this infection. In people who have recovered from tularemia immunity is elaborated of even higher strength than in those vaccinated. This may be judged by the more active immunological reactions as well as by the greater duration of immunity in those who have had the disease than in inoculees. However, in those inoculated the strength of immunity is quite adequate to protect against disease occurring from infection under ordinary epidemic conditions and, as practice has shown, immunity at this level can be maintained in the majority of inoculees for five-six years or longer (see Chapter X).

Other Methods of Vaccination. As is well known, in practice the most common method now is the percutaneous method of vaccination against tularemia. Aside from the simplicity of its application its important advantages are the following: 1) the possibility of recording accurately the "take" of the vaccine according to the skin changes and, by the same token, checking on the quality of the inoculations given; 2) the safety of the method in the case of an error in dosage. Nevertheless, along the line of theoretical studies, data obtained

in experiments on laboratory animals on the elucidation of the characteristics of the vaccine process after other methods of injecting the vaccine, such as aspiration, intranasal and alimentary, deserve attention.

In the experiments of R. A. Savel'yeva and G. P. Uglovoy on guinea pigs the possibility was shown of creating immunity in animals by means of the aspiration administration of tularemia vaccine (spraying the suspension in the form of a mist in a special chamber). Thereby, considerable sensitivity of guinea pigs to pulmonary administration of vaccine was noted -- a dose of 10,000,000 microbes was lethal for a considerable number of the experimental animals, which died with signs of total pneumonia (red hepatization of almost all the lobes of the lungs), the specificity of which was shown by isolation of the original vaccine culture. Aspiration of a dose of 100,000 microbes of the vaccine was tolerated by the guinea pigs, but three-four-day fever and a temporary delay in weight gain were observed. To date it has been considered firmly established that a vaccine culture is avirulent for guinea pigs in doses under 1,000,000,000 microbes, but this opinion is based on data of subcutaneous or other methods of injection, with respect to which the body is better protected.

Guinea pigs vaccinated by aspiration withstood subcutaneous and intradermal infection with massive doses (10,000 and 1,000,000 microbes) of the virulent 503 strain on checking the immunity two months after vaccination in the great majority of cases; they withstood aspiration infection less well, and as a result of it survival of the guinea pigs was noted only after a dose of 100 MLC<sub>50</sub>, whereas when the dose was increased the guinea pigs died of tularemia in the majority of cases (Table 27).

Data presented in Table 27 clearly show the inadequate protection of the immune organism against pulmonary infection with a virulent culture as the result of the special vulnerability of the pulmonary tissue. In other experiments of R. A. Savel'yeva and G. P. Uglovoy it was noted that guinea pigs vaccinated by the pulmonary route are resistant to aspiration infection with a virulent culture to approximately the same degree as guinea pigs immunized subcutaneously or intradermally.

In the experiments of B. Ya. El'bert and T. I. Puchkova (see Puchkova, 1948) and then of M. M. Kirvel' (1953) in guinea pigs the possibility was shown of immunization of the animals by means of instillation of a suspension of tularemia vaccine into their nostrils. M. M. Kirvel' could not find any notable difference in the production of immunity and in its strength as shown by subsequent infection with a dose of 1,000 MLD of a virulent culture in the comparative experiment.

Table 27

Strength of Immunity to Subcutaneous, Intradermal and Aspiration Infection with a Virulent Culture (503) in Guinea Pigs Vaccinated by Aspiration One-Two Months after the Vaccination (data of R. A. Savel'yeva and G. P. Uglovoy)

① Доза заражения (микробные клетки)	② Способ заражения					
	③ подкожный		④ интратканевый		⑤ аспирационный	
	заражено свинцом ⑥	пало от ту- ляремии ⑦	заражено свинцом ⑥	пало от ту- ляремии ⑦	заражено свинцом ⑥	пало от ту- ляремии ⑦
100	11	1	12	0	13	1
10 000	11	1	12	1	8	5
1 млн ⑧	9	1	10	0	12	11
	31	3	34	1	23	17

1. Infective dose (microbes); 2. Mode of infection; 3. Subcutaneous; 4. Intradermal; 5. Aspiration; 6. Guinea pigs infected; 7. Number died of tularemia; 8. Million.

of percutaneous and intranasal vaccination of guinea pigs with whole vaccine and vaccine diluted 10, 100, 1,000 and 10,000 times. However, M. M. Kirvel' pointed out that she "is not in favor of recommending intranasal utilization of the vaccine instead of percutaneous", considering possible cases of inflammatory reactions in the lymph nodes of the cervical ring or chest.

The alimentary method of vaccination in tularemia was studied experimentally by B. Ya. El'bert (1945) and then by I. F. Mikhaylov (1952). In experiments on guinea pigs the latter made it clear that the animals have to be given much more vaccine for successful alimentary immunization than for subcutaneous immunization, but even such a massive dose as 100,000,000 microbes assures immunity of adequate strength in only 90 percent of the animals. Such a result cannot be considered happenstance, because it is well known that after the alimentary administration of tularemia bacteria survival of them is interfered with by the bactericidal effect of gastric juice (R. A. Savel'yeva, 1956).

None of the methods of administering the vaccine analyzed has any advantages over the percutaneous method with regard to the immunizing effect, and the alimentary method is even inferior to it. The aspiration method of vaccination proved to be dangerous in cases of overdosage of the vaccine and difficult to use. The intranasal method cannot be considered safe either if we consider the ease of overdosage from instillation of the vaccine into the nose. We share entirely the opinion of M. M. Kirvel' presented above and her arguments against the use of the intranasal method for the vaccination of people. An essential defect of all three methods of application of the vaccine which we analyzed is also the fact that they do not submit readily to observation of the success of the inoculation. Therefore, of all the possible methods the percutaneous method of vaccination against tularemia proposed by B. Ya. El'bert is the best with respect to being practical and safe to use and, at the same time, is highly effective in relation to the various modes of infection under ordinary epidemic conditions.

Revaccination. As is well known, a second effect on the body with the bacterial antigen usually leads to an intensification of the immunological reactions and immunity. Increase in the resistance is manifested particularly sharply in case the repeated effect is accomplished during the period of subsidence of the protective reactions occurring as the result of the previous immunization. This problem has been particularly well studied with respect to bacterial toxins, and appropriate schemes of repeated inoculations are widely used in practice. With respect to living vaccines, including tularemia vaccine, this problem has been inadequately studied; however, clarification of this problem is of indubitable interest for practical immunology.

In the experiments of A. L. Matskevich (1952) in guinea pigs it was determined that revaccinated animals have a more pronounced immunity and better withstand infection with a virulent strain than vaccinated animals. The author vaccinated and revaccinated guinea pigs percutaneously, using liquid egg yolk vaccine and dry vaccine of the NIEG type. Revaccination was accomplished in the guinea pigs six-21 months after vaccination, at which time the immunological reactions (allergy, agglutination) were in the period of subsiding, while the test infection with a virulent strain was given one-and-a-half-four-seven months after revaccination. Similar experimental results in guinea pigs were obtained in our laboratory by L. S. Matveyets (1960). The author used shorter revaccination periods (two-four months after vaccination) and injected the vaccine intradermally. With an infection given by way of checking the immunity to a virulent culture one-three months after revaccination a clinically overt disease was observed in only a few animals, but in the majority of cases this ended in recovery.

in contrast to the control group of guinea pigs (vaccinated once), which suffered notably more from the infection, and some guinea pigs died of tularemia. In the experiments of the same author guinea pigs revaccinated twice (at an interval of two months) showed an even greater immunity to infection with a virulent culture than animals revaccinated once. However, this immunity did not reach the degree which characterizes immunity in guinea pigs which have been infected with a virulent culture.

After revaccination of people in whom a strong immunity still exists (after vaccination), the skin reaction at the injection site of the vaccine usually appears 24 or 48 hours later and is basically of an allergic character. However, a vaccine process from the partial survival and multiplication of the vaccine may be partly superimposed on the allergic reaction (M. F. Shmuter, 1953; G. P. Uglovoy, 1953, and others). In this case, in the revaccinated persons an increase is noted in the immunological reactions, specifically an increase in the agglutination titer of serum as well as a prolongation of the duration of the immunity.

With the revaccination of immune persons the accompanying reactions were usually noted more frequently than after vaccination (G. P. Uglovoy, 1953; V. A. Yudenich, 1954). This phenomenon should be attributed to sensitization of the immune organism to the tularemia antigen. In persons who have completely lost their immunity at the time of revaccination inoculation is associated with the development of a vaccine process which occurs at the same time and with the same intensity as in persons being immunized for the first time (V. A. Yudenich, 1954; L. S. Matveyets, 1960). As far as can be judged by immunological reactions, immunity in these persons returns to the level reached at the time of the first vaccination. These problems will be analyzed in greater detail in Chapter X.

**Associated Vaccination.** Because inoculations against tularemia frequently are conducted in localities in which the population is subject to vaccination against other common infectious diseases the problem arises of the development of associated vaccines, which can considerably simplify the inoculation measures. The possibility of using associated living vaccines (with inclusion of the tularemia vaccine) was first pointed out by B. Ya. El'bert (1945) on the basis of experiments of simultaneous percutaneous vaccination of guinea pigs against tularemia and smallpox. Associated vaccination simultaneously against tularemia, smallpox, tetanus and the group of intestinal diseases is reported by V. G. Akimenko (1949); the author performed experiments on rabbits and guinea pigs; however, the details of the investigation made were not published.

Simultaneous vaccination against tularemia and brucellosis was subjected to a very detailed study experimentally. Experiments on guinea pigs showed that after subcutaneous or percutaneous vaccination with a mixture of tularemia and brucellosis vaccines the local and general reactions do not exceed those in control guinea pigs inoculated with a single vaccine. Both vaccines in the mixture produce corresponding immunological reactions in the guinea pig organism (skin allergy, antibodies) as well as the production of an immunity of adequate strength both to tularemia and brucellosis, which is not particularly inferior in any way to that produced when the vaccination is given with each vaccine separately (V. G. Pilipenko and coauthors, 1955, 1956; Ye. A. Gubina, 1957; K. N. Shlygina, 1958, and others). In tests made on people harmlessness and good acceptability (by the skin inoculation test) and immunogenicity (by seroallergic tests) of the associated vaccine were found against tularemia and brucellosis (R. S. Amanzhulov, 1956; R. S. Amanzhulov and M. M. Rementsova, 1958; Ye. A. Gubina and G. P. Uglovoy, 1958, and others). In experiments on guinea pigs the possibility was shown of simultaneous immunization with living vaccines against tularemia and plague (N. F. Kalacheva, 1958) as well as against tularemia, brucellosis and plague (N. K. Vereninova and coauthors, 1958; V. G. Pilipenko and coauthors, 1959) but not against tularemia and anthrax. The production of immunity to this latter infectious disease was definitely depressed with associated vaccination.

What has been presented indicates the promise of study and practical testing of associated living vaccines. Specifically, attention should be directed to the possibility of making up combined vaccines with inclusion of tuberculosis vaccine.

Vaccination Under Conditions of Radiation Injury or Other Effects (Experimentally). In the experiments of R. A. Savel'yeva on white mice it was determined that irradiation of the animals with a dose of 350 r markedly aggravates the course of vaccine tularemia. When the climax of radiation sickness coincides with the vaccine process being produced by a dose of 10,000 microbes of the 15-reconstituted strain all the experimental mice die. If radiation injury occurs at the end of the vaccine process, part of the mice survive, and immunity is found in them which is adequate to withstand subcutaneous infection with 1,000 MLCD of a virulent tularemia strain. Aggravation of the vaccine process in mice with radiation sickness was also noted in his experiments by A. S. Shevelev (1958). According to the data of R. A. Savel'yeva, irradiation of guinea pigs with a dose of 200 r did not aggravate the course of the vaccine process, but in the animals a partial depression of immunological reactions was noted, and a considerable

part of the guinea pigs died of tularemia on test infection with a virulent culture. In experiments of V. M. Zhidovtsev (1958) a notable reduction of agglutination titers of the serum after x-ray irradiation of the animals was noted in rabbits and guinea pigs immunized with living tularemia vaccine. M. P. Tereshchenko noted a marked aggravation of vaccine tularemia in white mice under the influence of cortisone. N. K. Vereninova and N. F. Kalacheva (1954) noted an intensification of the immunizing effect of tularemia vaccine with simultaneous injection of hyaluronidase in experiments on mice, and M. M. Faybich (1959) found the same effect from inoculation of mice with a vaccine suspension in 0.2 percent agar solution as well as in a solution of one percent gelatin and other colloids.

#### Immunity from the Injection of Killed Corpuscular and Chemical Vaccines

(The reader can become acquainted in greater detail with Soviet work prior to 1952 done on killed tularemia vaccines in the monograph of I. N. Mayskiy (1953)).

Clarification of the immunizatory effect of tularemia bacteria killed by one method or another for a long time attracted the main attention of research workers. However, as is well known, this trend did not bring any perceptible practical result in the form of a preparation which would assure effective immunization of people against tularemia. Nevertheless, we consider it useful to discuss briefly the main stages of the work done and the current status of this problem, considering the fact that interest in the study of "chemical" vaccines is continuing, particularly in the United States.

Killed corpuscular vaccines obtained by means of inactivation of virulent bacteria by heating, treating with phenol, formalin and other substances as well as lysates from bacteria have proved to be practically ineffective on testing in such animals highly sensitive to tularemia as white mice and guinea pigs (Francis, 1929; B. Ya. El'bert and N. A. Gayskiy, 1941; N. K. Vereninova and coauthors, 1943; N. D. Altareva, 1949). Good immunization results in guinea pigs and white mice with phenol and formalin vaccines were reported by Kudo (1934), but the value of his experiments is very relative, because immunity in the experimental animals was checked by means of infection with partially attenuated strains of *B. tularensis* [in this text actually *F. tularensis* is the term used]. This comment should be made also with respect to the first works on killed vaccines by Soviet investigators G. Ya. Sinay (1935) as well as L. M. Khatenever and L. A. Levchenko (1935, 1938).

Some authors (Downs and others, 1947; Coriell and co-authors, 1947) used a culture of *B. tularensis* in the yolk sacs of chick embryos for the purpose of obtaining an antigen and for preparing killed vaccines, believing that the vaccine can be more effective when made of bacteria grown in living tissues than when coming from a culture on synthetic nutrient medium which, however, was not confirmed in experiments. In testing some killed vaccines on domestic rabbits results which were partially positive were obtained in the immunization of the animals to subsequent infection with a virulent culture (*B. Ya. El'bert* and *N. A. Gayskiy*, 1941; *P. N. Burgasov*, 1951, and others).

*Foshay* (1950), who did considerable work on the problem of vaccine prophylaxis of tularemia and who proposed a "nitrogen" vaccine, noted the uselessness of laboratory testing of vaccines on experimental animals which do not have a natural resistance to tularemia (that is, white mice, guinea pigs, etc.) and the suitability of white rats for this purpose. The possibility of immunization of white rats with killed vaccines is also pointed out by *Larson* (1945) and *Downs* and coauthors (1947). In the American literature the results of testing killed corpuscular vaccines on people were published in the works of *Foshay* and coauthors (1942), *Kadull* and coauthors (1950) and others; however, the prophylactic effectiveness of the vaccines tested was low. Among the Soviet authors killed vaccine was tried out on people by *L. M. Khatenever* and coauthors but also with a slight effect. For more details on this subject see the next Chapter.

Along with killed corpuscular vaccines or lysates of them a study was also made of the immunizing properties of antigenic complexes extracted chemically from the microbe. *I. N. Mayskiy* (1953) points out the successful vaccination of guinea pigs with complete antigen obtained by *G. K. Shipitsina* from a virulent culture by the *Boivin* method. After triple immunization the animals withstood infection with 1,000 MLD of a virulent strain of *B. tularensis*. The immunizing effect of the whole antigen in experiments on guinea pigs is also reported by *G. K. Shipitsina* (1955). She obtained the best results from subcutaneous injection of the preparation in a lanolin depot. Data on the chemical composition of the antigen are reported in Chapter III. In the experiments of *I. S. Tinker* and coauthors (1955) rabbits, guinea pigs and, partly, white mice immunized intradermally with the whole antigen extracted from a tularemia culture by the *Boivin* method, showed resistance to infection with doses of a virulent culture of *B. tularensis* known to be lethal. The protection of the animal organism conferred by the whole antigen was brief; for example, guinea pigs lost immunity three months after vaccination. *Bell* and coauthors (1952) as well as *Ormsbee* and coauthors (1955) immunized white mice



with an antigen soluble in physiological saline solution which was extracted from virulent bacteria by the Larson method (extraction in the presence of sulfuric ether) and then subjected to purification and concentration (for more details see Chapter III). After a single or double subcutaneous immunization with this preparation the majority of white mice withstood a test infection with one-1,000 MLCD of strains with reduced virulence for rabbits (with respect to pathogenicity for rabbits these strains were approximately the same as Soviet strains but they were fully virulent for white mice and guinea pigs ( $LD_{50}$  = one bacterium)), whereas the immunity of the mice to the fully virulent American strains was inadequate, and the animals died of tularemia.

The slight immunizing effect of the antigen obtained by the Larson method was noted in experiments on white mice by A. I. Belkin and Ye. A. Petrosyan (1953). According to the data of O. S. Yemel'yanova, the antigen from virulent bacteria obtained by G. K. Shipitsina by the Larson method possessed slight immunogenic properties in experiments on white mice but the preparation proved to be effective enough for the immunization of white rats after a single injection to protect the animals against death from infection with 1,000,000,000 microbes of the virulent 503 strain.

In summary, it may be noted that the results of testing of the antigenic complexes on laboratory animals and particularly on those models sensitive to tularemia such as white mice and guinea pigs were somewhat better in a prophylactic respect than the killed corpuscular vaccines, which indicates a certain degree of progress in the general development of this problem. The immunizing effect of vaccines of this type could be increased, evidently, by more complete extraction of the Vi antigen from the microbes and less denaturation of it as well as by greater concentration of it in the preparation used for immunizing animals. Thus, in the experiments of Bell and Larson and co-authors with a single immunization of mice a quantity of antigen was used which had been extracted from one milligram of dry bacteria, which is approximately equal to 5,000,000,000 microbes (according to the GKI standard). This quantity far exceeds the doses which were given to white mice in the form of corpuscular vaccines in the experiments of authors quoted above.

The development of "chemical" vaccines cannot be considered complete, and methods of obtaining them cannot be considered exhausted. This trend should attract the attention of workers further. It should be noted only that all the antigens obtained so far in the laboratory experiment are clearly inferior to the living vaccine in their effectiveness, without mentioning the incommensurate simplicity of using the latter in people because of the percutaneous method of appli-

cation and the fact that only one injection is needed. It is very possible that the reduced immunizing activity of killed vaccines is associated with the fact that in the antigenic substances existing in them the colloidal state is disturbed to one degree or another. Conversion of the antigens to complete colloidal compounds would probably increase their immunogenicity.

### Passive Immunity

Study of the protective properties of antitularemia immune sera was conducted abroad and in the USSR and had the aim of developing therapy for tularemia. This study has not given the proper practical result and now has been abandoned, particularly in connection with the appearance and clinical application of such powerful therapeutic measures as antibiotics, which have proved to be highly effective against tularemia also.

Experimental investigations conducted have shown that in such models as white mice, very sensitive to tularemia, it is very hard to demonstrate the protective effect of immune sera (horse, goat, ram, rabbit), with the exception of those cases in which infection of the animals is accomplished by strains of *B. tularensis* which have been attenuated to varying degrees (Kudo, 1934) or when the serum is injected simultaneously with a very small infective dose of bacteria (B. Ya. El'bert and N. A. Gayskiy, 1941; N. K. Vereninova and coauthors, 1943). Experiments on guinea pigs were also unsuccessful (N. A. Popov, 1943; I. N. Mayskiy, 1953). Notably better results were obtained from testing sera on animals less sensitive to tularemia -- domestic rabbits (A. A. Miller and N. K. Grzhebina, 1937) and white rats (Foshay and coauthors, 1947). In experiments on rabbits B. Ya. El'bert and N. A. Gayskiy showed the possibility of extinguishing the hyperergic reaction (Schwartzmann phenomenon) as well as the cutaneous necrotic reaction by means of immune serum. The antinecrotic function was most pronounced in the sera of rabbits which had had tularemia, then in the sera of rams, and then horses. The absence of bactericidal and bacteriostatic properties in immune sera (B. Ya. El'bert and N. A. Gayskiy, 1941; N. K. Vereninova and A. N. Kursheva, 1947) clearly shows that the preventive effect of sera, in contrast to antibiotics, can be expressed only in those cases where the natural defensive forces of the macroorganism are not too much suppressed by the developing infection. Experiments on relatively resistance rabbits and white rats as well as the results of testing on people (see below) confirm this well.

Immune sera obtained by acting on animals with a living

virulent culture were more effective than sera obtained by means of immunization with suspensions of killed bacteria, which clearly indicates the greater antigenic completeness of the living culture. Relatively modest results, obtained by the majority of authors in the testing of immune sera on laboratory animals, did not stop various investigators from attempts at using serum treatment for tularemia in people. This occurred until antibiotics appeared. Abroad, the largest number of studies on serotherapy of tularemia was made in the United States by Foshay (1932, 1940); in the USSR the pioneers in this trend were A. A. Vol'ferts and V. I. Gorokhov (1935); later, N. A. Popov (1940), N. K. Grzhebina (1941) and L. K. Denisenko (N. A. Popov, 1943). Without going into details, we should like to note that with timely (early) application immune sera in a number of cases have exerted a notable therapeutic effect, expressed in an improvement in the patient's feeling of well-being, shortening of the febrile period and limitation of the local inflammatory process. Foshay (1940) was able to note a reduction in the mortality rate among patients treated with serum.

#### Immunological Reactions and Their Relation to Immunity

The immunizatory process in tularemia is accompanied by allergization of the organism, the formation of specific antibodies and the increase in phagocyte activity. For the purpose of demonstrating allergic reactivity of the organism the allergic skin test is used. With the aim of detecting antibodies usually recourse is had to the agglutination test, but the complement-fixation test, precipitation test and hemagglutination test can also be used. For the purpose of detecting opsonins and determining the activity of phagocytes recourse is had to the opsonocytophagic test (see Chapter VIII). The development of tests by means of which the antibodies can be detected in the immune organism does not, however, violate the principle of unity of these antibodies (L. A. Zil'ber, 1948).

As is well known, in various infectious diseases the interrelationship between the actual state of immunity and the immunological reactions observed differs, although the development of these latter are most closely connected with changes in the body occurring under the influence of pathogenic agents or their activity products. In the opinion of B. Ya. El'bert and N. A. Gayskiy (1941), in tularemia the greatest degree of correlation is found between immunity and allergy. These authors have repeatedly emphasized the inseparable connection between immunizing and allergizing properties of the tularemia pathogen with the leading significance of the H component of the microbe

cell (that is, of the Vi antigenic complex). Subsequent investigators also indicated the close connection between allergy and immunity in tularemia (I. N. Mayskiy, 1953; T. A. Kalitina, 1953; V. A. Yudenich, 1954, and others). Existing data on the development of the allergic reactivity in the course of immunization as demonstrated by the skin test show that this reactivity is expressed most strongly and for a long time in the organism as the result of the effect of a virulent culture on it, that is, by the strongest and most complete stimulus in an antigenic respect; however, thereby the strongest immunity is attained. A more moderate and somewhat less prolonged allergization of the body corresponds to a relatively weaker and less prolonged immunity produced by living vaccine.

According to the data of T. A. Kalitina (1953), skin allergic sensitivity in those guinea pigs which have recovered from tularemia is approximately 100 times greater than in those immunized with the living vaccine. By means of using fractional doses of ordinary tularin this author established the fact that people who have recovered from tularemia react to the intradermal injection of 1/100,000 or 1/10,000 of the dose of preparation, whereas those immunized with living vaccine react to 1/1,000 of the dose, but in a number of cases, to 1/100 or even lesser dilutions of tularin. Such considerable differences in the allergic skin reactivity between those people who have recovered and those who have been vaccinated create certain difficulties in selection of the preparation which would not cause excessive local and general reactions after intradermal injection of those who have recovered, and at the same time, would be fully adequate for demonstrating allergic reactivity in those vaccinated. Percutaneous application of the allergen with an appropriate selection of the effective dose solves this problem quite satisfactorily.

Killed corpuscular vaccines, being least immunogenic, do not possess allergizing properties (B. Ya. El'bert and N. A. Gayskiy, 1941) or show them to a minimum degree (N. D. Altareva, 1949). In ascribing great importance to the allergic skin test as an index of immunity in tularemia N. A. Gayskiy in his tests on people with living vaccine used this reaction as a basis for the selection of the vaccinating dose. He believed that in tularemia "allergy and immunity follow each other as a shadow follows its object" (N. A. Gayskiy, 1946). The close connection between the immunogenic and allergenic properties of the tularemia microbe is indubitable. However, as has been noted in Chapter VIII the development of immunity and its subsequent preservation cannot be detected in all species of animals by means of the intradermal allergic test. In a number of species of small rodents this test is macroscopically negative in the presence of immunity of

great strength after recovery from tularemia. What has been stated does not rule out the probable existence of allergy in these animals, that is, the capacity of a rapid response reaction of the body to a second injection of the antigen, but evidently the methods of detecting it should be different from the skin test.

It would be an error to consider that the allergic reactivity is characteristic only of the skin of an organism immune to tularemia. Allergic reorganization encompasses the entire organism, which is indicated by the active general reactions in the form of a temperature elevation, malaise, etc. which develop in immune people after the injection of appropriate doses of the antigen intradermally, subcutaneously and particularly intravenously. Vigorous general reactions of an allergic nature have been observed repeatedly in patients with tularemia after the use of vaccine therapy. The entrance of the antigen into the respiratory tract of a sensitized organism causes an allergic reaction of a general nature, which was noted even by N. A. Gayskiy (1943) and observed repeatedly in our laboratory.

In guinea pigs a complete correlation between skin allergy and immunity is observed only after recovery from tularemia, which produces immunity of great strength. In guinea pigs immunized against tularemia with living vaccine the connection between immunity and allergic reactivity is relative, which corresponds to an immunity of moderate strength created in guinea pigs under the influence of vaccination (T. A. Kalitina, 1953). The differences between the allergic test and the actual state of immunity increase in frequency with increase in the length of time after vaccination.

T. A. Kalitina noted cases in which the skin allergic reactivity of guinea pigs was preserved but the immunity was inadequate to prevent death from tularemia with a test infection of 100 MLCD of a virulent strain, although the survival time was prolonged somewhat. Similar data occur in the work by N. D. Altareva (1949).

The most complete correlation between immunity and skin allergic reactivity is observed in people, not only after recovery, but also as the result of immunization with living vaccine. This is associated with the fact that the possibility of mobilization of the natural defense mechanisms against tularemia as well as the ability to be sensitized to tularemia antigens are much greater in the human body than in the guinea pig organism and in the bodies of a number of other animals. However, rare cases of differences between the allergic skin reactivity to tularin and the actual state of immunity can also occur in people, we believe.

In dealing with the mechanisms of the allergic reaction we may refer to the common opinion that underlying it is the interaction

between the allergen and the antibody. The latter occurs inside the cells of the macroorganism, interfering with cell metabolism and leading to the increased production of histamine and other stimulants in the tissues. After the intradermal injection of a corpuscular antigen (tularin) the reaction in the form of hyperemia and infiltration of a certain area of skin develops in the sensitized organism usually no earlier than 12-18 hours afterwards and reaches a maximum after 36-48 hours. Evidently, this time is necessary for the cellular digestion of bacteria injected into the skin and the liberation of a quantity of antigenic substances which after interaction with the antibodies would produce the appropriate cell reactions (see above). After percutaneous injection of the antigen the time of appearance of the skin reaction can drag out to 24-48 hours, particularly in vaccinated persons. After the intradermal injection of soluble antigenic complexes ("tuallergen") into the sensitized organism the allergic reaction develops much more rapidly for example, in people it appears as early as several minutes after the injection (I. N. Mayskiy and G. K. Shipitsina, 1952) which indicates a rapid interaction between the substances injected and antibodies of the immune organism. This reaction proceeds in a manner similar to the skin test with specific pneumococcus polysaccharide during the period of convalescence from pneumonia: the vesicle-reddening reaction occurs immediately after injection of the antigen. (Sh. D. Moshkevskiy, Allergy and Immunity, 1947, page 60). The rate of occurrence of the skin allergic test after injection of "tuallergen" is similar to that in the precipitation test in vitro. In this case the intensity and specificity of the allergic skin test of an organism immune to tularemia should primarily be associated with the polysaccharides as being the most active substances in the precipitation reaction. According to the data of G. K. Shipitsina (1954), the necrotizing effect from performance of the allergic test is associated with the lipids of the whole antigen. A similar accelerated reaction occurring as early as the first few minutes after the injection is observed after the intradermal injection of specific hyperimmune serum into patients with tularemia. The test can be positive as early as several hours after the onset of the disease (Forshey, 1936, 1940). In this case the serum injected enters into a reaction with the antigen which is in the patient's body (the so-called "reverse allergic reaction").

It should be kept in mind that the intradermal tularin test itself is not immaterial to the body, since the antigen of the pathogen is injected into the body. For example, in guinea pigs which are not immune to tularemia a single intradermal injection of tularin brings about the rapid appearance of a small quantity of agglutinine in the blood serum, which disappears after two or three weeks (T. A. Kalitin,

1953). There are also indications of the occurrence of agglutinins in non-immune people after intradermal tularin tests performed twice (L. M. Khatenever and coauthors, 1935). A single intradermal injection of tularin into guinea pigs and white mice can in occasional cases create a temporary resistance in them to subsequent infection with several MLCD of a virulent tularemia culture, whereas a similar injection of this preparation into white rats creates the same resistance in the majority of animals (T. N. Dunayeva, 1959). These data indicate the need of cautious utilization of the intradermal test for checking animals in an immunological experiment, because the immunization processes being studied can be thereby distorted to one degree or another.

In summing up what has been stated it may be noted that in tularemia the skin allergic test can be considered a comparatively reliable criterion of immunity in man and, to a certain degree, in various species of animals, but it is not of general significance for all species of animals. It should be taken into consideration that different states of the body and of its central nervous system can exert an influence upon the skin allergic sensitivity. Thus, N. A. Gayskiy (1944) noted that hibernation in sousliks and tarbagans reduces their allergic reactivity. The skin allergic sensitivity of guinea pigs immune to tularemia can be reduced in cases of gravidity, in the case of a pneumococcal infection, etc. (T. A. Kalitina, 1953).

There is an indication of the early extinction of allergic reactivity and the disappearance of agglutinins from persons inoculated against tularemia suffering from pulmonary tuberculosis in the stage of decompensation (T. A. Rubina, 1951). I. F. Mikhaylov (1951) directed attention to the possibility of reproduction of the allergic skin test by the conditioned-reflex method in guinea pigs immune to tularemia. (In the experiments of this author immunity to tularemia in guinea pigs was achieved by infection of them with a virulent culture after preliminary immunization with living vaccine). He used a puncture with a needle and subsequent mechanical stretching of the skin with physiological saline solution, combining this stimulation with the specific action of tularin on the skin receptors. After five such combinations, in the sixth injection he gave the animals physiological saline solution instead of the specific agent, tularin. Thereby, at the injection site of physiological saline solution on the next day hyperemia was noted in the area of skin with an infiltrate of the underlying tissue which in a number of cases were of the same intensity as in response to the previous injections of tularin. Evidently, in this case the reaction was of the conditioned-reflex type without participation of a specific agent. The experiments of I. F. Mikhaylov were repeated on

guinea pigs by T. A. Kalitina (1953) and independently of her by V. A. Yudenich (1954), and they were fully confirmed. T. A. Kalitina established the fact that the skin reactivity occurring in response to a conditioned stimulus is ephemeral, and in the animals it is completely lost as early as after two weeks. We should like to note that the possibility of obtaining a positive allergic skin test by the conditioned-reflex method in response to the injection of physiological saline solution was shown by Malis (1935) before Mikhaylov in experimental tuberculosis of rabbits.

In guinea pigs immune to tularemia it is possible to desensitize the skin to this preparation by means of frequent intradermal injections of tularin given over a long period of time (I. F. Mikhaylov, 1951; T. A. Kalitina, 1953); however, the latter does not bring about a loss of immunity in the guinea pigs (T. A. Kalitina). The possibility of desensitization of the skin in people immune to tularemia by repeated injection of the antigen has been indicated by Foshay (1940). A. V. Mashkov (1946) notes that after a single intravenous injection of 2,000,000,000 killed tularemia bacteria (diagnosticum) into immune rabbits the skin reactivity to tularin was completely extinguished and there was a considerable reduction of the serum agglutination titer, but after two weeks the allergy and the agglutinins were restored. According to the observations of T. A. Kalitina (1953), after repeated injections of tularin into immune guinea pigs but after long intervals, for example, every three months, the skin reactivity to tularin increased in a number of cases.

What has been presented shows that the skin allergic reactivity of the immune organism is subordinate to the general biological law of excitation-inhibition. This is also confirmed by the possibility of extinction of the allergic skin reaction in guinea pigs immune to tularemia by means of putting them to sleep with anesthetic agents (I. F. Mikhaylov, 1951; T. A. Kalitina, 1953; A. S. Shevelev, 1954).

Along with the allergic reaction N. A. Gayskiy (1948) ascribed great importance to the opsonocytophagic test as an index of immunity in tularemia. He was able to find a high percentage of correlation between both reactions in the guinea pigs immunized with living vaccine as well as in people, which was confirmed in the subsequent investigations of I. S. Tinker and A. N. Yelfimova (1956) as well as those of R. Ya. Bondar' (1957). Thereby, it was noted that the phagocytic activity of blood leukocytes in non-immune people, guinea pigs and rabbits is best expressed in response to avirulent bacteria; in immune people or animals, in response to virulent or vaccine bacteria (I. S. Tinker and A. N. Yelfimova, 1956).

In the opinion of a number of investigators, antibodies and



particularly agglutinins can be regarded only as a relative index of immunity in tularemia (N. A. Gayskiy, 1943; L. M. Khatenever, 1943; I. N. Mayskiy, 1953, and others). Factual material accumulated at present on the agglutination test and other serological tests in tularemia shows that the antibodies appear quite regularly in the macroorganism after the effect of both virulent and variously attenuated tularemia bacteria, living or killed, as well as antigenic complexes extracted from the microbe. However, the quantity of antibodies and the length of time they circulate in the blood depend on the strength (quality) of the antigenic stimulus used, its dose, method of application, etc. as well as on the species characteristics of the macroorganism (and in a number of cases also the individual characteristics) on which the antigenic effect has been exerted. The qualitative characteristics of the antibodies formed and particularly the interrelationship between the Vi and O antibodies (see Chapter III) should depend on the qualitative composition of the antigenic complexes causing the immunizatory process. This problem has not been studied in tularemia, but without the solution of it a number of very important aspects of immunology of this infectious disease remain unclear. Under otherwise equal conditions the highest level of antibody production in the body occurs when the body is acted on by a virulent tularemia culture; a moderate level, after vaccination with a living attenuated culture; and a low level, after the effect of killed vaccine. The rules and regulations, therefore, are the same as in the formation of the allergic skin reactivity during the course of the immunizatory process.

For example, during the period of maximum antibody production in people who have recovered from tularemia the agglutination titers amount to 1:400-1:800 but can reach 1:1600 and 1:3200, whereas in people immunized percutaneously with living vaccine these titers usually amount to 1:80-1:160 and rarely exceed 1:320. The antibodies formed react in vitro with the virulent or vaccine culture chiefly in a manner similar to stable agglutination, to which attention was directed by B. Ya. El'bert and N. A. Gayskiy (1941).

In contrast to skin allergy the antibodies are found after immunization in the most varied species of animals, but the level attained is different (see Chapter VIII). After reaching the maximum the quantity of antibodies then decreases and then they remain at a relatively low level in the body. During this period usually cases of a discrepancy between the existence of antibodies (as demonstrated in the agglutination test) and the actual state of immunity begin to be noted, the more often the longer the period which has elapsed since the time of immunization.

In people inoculated with living vaccine, during the first

year after immunization usually both reactions are positive -- the allergic and agglutination reactions. In subsequent years, when the agglutination titers become low, more often it is possible to find only the former reaction, for example, after three years, according to the data of V. A. Yudenich (1953), the allergic reactivity to tularin was demonstrated in 84.2 percent, and agglutinins (in a dilution of 1:5-1:80) were found in 61.4 percent of the inoculees.

Among practising physicians the opinion is common that in tularemia the development of allergy outstrips the appearance of antibodies. This opinion is based on the fact that in diagnostic practice the skin tularin test becomes positive sooner than the agglutination test. However, the latter is not an adequately sensitive method of detecting antibodies, particularly if minimum serum dilutions are neglected in its performance (1:5-1:10). It is well known that in the complement-fixation test antibodies can be found earlier than in the agglutination test in a number of cases (see Chapter VIII). Therefore, a certain gap in the time between the detection of allergic reactivity in the body during the course of the immunizatory process and the detection of antibodies, noted in clinical practice, does not reflect the true state of affairs. Early appearance of agglutinins, preceding the development of the skin allergic sensitivity (to the doses of tularin used) was noted in their experiments by A. V. Mashkov (1946) and I. N. Mayskiy (1953): the former, after infection of rabbits with a virulent culture; the latter, after immunization of guinea pigs with living vaccine. Therefore, the opinions stated by I. N. Mayskiy to the effect that "immunological changes in the body ... are essentially the same, and the impossibility of simultaneous detection of them by the methods of investigation used is explained by the different quantitative manifestations of these changes in the immunized organism" should be considered justified. To what has been stated the probable imperfection of the methods which we are using at the present time for the detection of immunizatory processes in the body should be added.

The condition of the central nervous system exerts an effect on the development of immunological reactions, which was demonstrated by means of observations on the tularemia vaccine process in persons with mental disorders. Thus, in certain forms of schizophrenia (for example, in a catatonic stupor) as well as in the presence of signs of parkinsonism the immunological indices (allergy, antibodies) in response to vaccination are notably reduced by comparison with normal. However, in feeble-mindedness the immunological reactivity is no less than that of normal subjects (L. S. Matveyets, N. G. Olsufyev, Yu. A. Il'inskiy, N. M. Zharikov and O. V. Kerbikov, 1957). These data indicate that a functional abnormality of subcortical activity

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plays an essential part in the change in the immunological reactivity of the body. The observations presented show that reduction of the effect of vaccination against tularemia can be expected only in persons who have definite and quite severe pathological changes in the central nervous system.

#### Immunity Mechanisms in Tularemia

B. Ya. El'bert and N. A. Gayskiy believed that in tularemia a non-sterile immunity occurs. [A sterile immunity is one in which there are no living bacteria of the kind which produced the immunity in the body]. In their opinion, the presence of living bacteria in the macroorganism is an inevitable condition for the maintenance of the immunity on the proper level (the premunition principle of Sergeant-Donatienne). The impossibility of creating immunity by means of killed vaccines was considered one of the main proofs of this viewpoint. In the opinion of B. Ya. El'bert and N. A. Gayskiy, the site of localization of tularemia bacteria in the immune organism is chiefly the bone marrow.

I. S. Tinker and M. S. Drozhevskina (1949), on the basis of experiments on rabbits, indicated the possibility of a prolonged existence of virulent bacteria in the immune organism. However, the data of these authors cannot be considered convincing because of their application with the aim of detecting the microbe by the "multipassage" method. Verification experiments on rabbits, performed in our laboratory, did not confirm the observations of I. S. Tinker and M. S. Drozhevskina. L. M. Khatenever (1943, 1946) and then V. P. Dzhanpoladova (1948), P. N. Burgasov (1951), I. N. Mayskiy (1953) and N. G. Olsuf'yev (1953), based on numerous experiments on different species of animals, expressed opposite opinions as to the sterilizing nature of the immunity in tularemia, its regular transition from the infectious phase to the post-infectious phase during the course of development. Here, as well as in Chapter IV, we have repeatedly presented experimental materials confirming this viewpoint and coinciding with similar data by P. F. Zdrovovskiy and his co-workers with respect to brucellosis and other infectious diseases. Particularly convincing are the experiments on animals of group II (white rats, rabbits, etc.) in which, as the result of their lesser sensitivity to infection, the mechanisms of defense are rapidly mobilized and the body is rid of virulent tularemia bacteria in quite a short time (usually in one-two months) during the recovery process, while the immunity acquired is then preserved for a long time. Guinea pigs and white mice, because of their high degree of sensitivity to tularemia, are not such good

models for these experiments, but if the experiments are performed in a certain way it is possible to create a very strong immunity and note the transition of this infectious phase into a post-infectious one. Numerous observations attest to the fact that during the course of development of immunity the regional lymph nodes (and lungs) usually become free of tularemia bacteria last, but the bone marrow does not. Various cases of lingering bacterial carriage indicate only the imperfection of protective mechanisms and cannot serve as proof of the non-sterile immunity in tularemia. The possibility of reproduction of immunity to tularemia in animals by means of the injection of the so-called "chemical" vaccines deprives one of the many arguments of proponents of the non-sterile immunity of its significance.

In dealing with the intrinsic mechanisms of immunity in tularemia B. Ya. El'bert and N. A. Gayskiy (1941) emphasized the fact that resistance of the immune organism to reinfection is associated with specific allergic reorganization of cells of the latter, directed at rapid elimination of the pathogen from the focus. In the opinion of N. A. Gayskiy (1943), immunity in tularemia is associated with an allergic reorganization of the body; the appearance of agglutinins in the blood should be regarded only as proof of the fact that infection has occurred which has no direct bearing on the production of immunity. The secondary role of antibodies in immunity to tularemia was pointed out by I. N. Mayskiy (1953) and A. V. Mashkov (1952). Thereby, they referred to the fact that in the remote periods after recovery or vaccination agglutinins can no longer be found in the blood serum, but the actual state of immunity (that is, resistance to reinfection) continues to be maintained. At the same time, experiments on vaccination with killed corpuscular vaccines show that despite the appearance of agglutinins in the sera of animals the allergic reaction and pronounced immunity (to infection) are absent in them. Both authors believe that underlying immunity in tularemia is an increase in the reactivity of the body, that is, in the ability of the latter to respond with accelerated tissue reactions, at the time of a second encounter with the pathogen, leading to a restriction of multiplication of the microbe and destruction of it. Both authors also direct attention to the significance of the regulatory effect of the central nervous system in the change of the immunological reactivity of the body.

The arguments of the authors mentioned above concerning the secondary role of antibodies in the mechanisms of immunity to tularemia cannot be considered convincing. The fact that antibodies are not found in the blood of an immune organism does not mean that they are entirely absent from it, because they may be contained in the cells (sessile antibodies). This may be indicated indirectly by the skin

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allergic reactivity observed in the immune organism, the detection of which reactivity is based on the reaction between antigen and antibody. For the purpose of detecting the antibodies the authors usually made use of the agglutination test, which is far from perfect for these purposes. Thereby, no consideration was given to the antibody pattern in the sera being studied or the quantity of anti-Vi components in them, particularly after immunization with killed corpuscular vaccines. The problem of antibodies in immunity to tularemia needs checking with the use of more perfect methods of detection (the hemagglutination test, method of quantitative precipitation, etc.). Specifically, a check on the statement that precipitins better reflect the activity of sera immune to tularemia than do agglutinins is interesting (N. K. Vereninova, 1947; Foshay and coauthors, 1947). We should like to note that considerable progress in the study of the role of various antibodies in immunity to plague was made as the result of using hemagglutination.

What has been stated gives no basis for accepting a function of direct protection for antibodies. Without possessing this, antibodies, reacting with the antigen, only specifically direct the natural defense forces of the body, which in themselves possess no specificity of action (L. A. Zil'ber, 1948, 1958; V. S. Gostev, 1954), against it and its carrier -- the bacterial cell. This has been confirmed by the latest investigations on the characteristics of formation of antibacterial immunity after the effect of ionizing radiation on the body, which shows that antibodies participate in protection of the body only when natural nonspecific mechanisms of protection are not injured (V. L. Troitskiy and coauthors, 1956). Similar rules and regulations have been established with respect to antitoxic diphtheria immunity in experiments on guinea pigs, including reproduction of it by the passive method (D. R. Kaulen, 1956). In the light of these data the failure of transmitting passive immunity to such animals, so highly sensitive to tularemia as guinea pigs and white mice, in which during the course of development of the infectious process caused by fully virulent strains of bacteria the natural defense mechanisms are paralyzed very early and are then injured (see Chapter IV), becomes understandable. In animals less sensitive to tularemia, for example, white rats or rabbits the natural defense mechanisms suffer less, and in these cases serum therapy under certain conditions can be effective, just as in the case of man.

A. V. Mashkov (1952) points to inflammation and phagocytosis as specific methods of protecting the organism immune to tularemia against reinfection. Of the natural humoral factors note should be made of complement, opsonins, and properdin, but evidently the list is not limited to this. It is fitting to mention that the allergic skin reaction developing in an organism immune to tularemia after the

injection of killed or living tularemia bacteria has features of specific inflammation (granulomatosis), and underlying it it has a histiocytic (macrophagic) character (T. A. Kalitina and A. P. Gindin, 1956), that is, it is essentially protective.

With respect to phagocytosis it should be noted that in the case of immunity to tularemia it cannot be studied with appropriate completeness, although the significance of it is indubitable and very important. Investigations on this problem by V. P. Dzhanpoladova (1948) are not convincing, because the author did not have at his disposal selective staining for the tularemia bacteria, which, given the very small size of the latter does not permit reliable differentiation of them from granular inclusions of leukocytes in various pathological conditions of the body. In this connection staining by the Romanowsky-Giemsa method cannot be considered adequate. Observations obtained with the use of the opsonocytophagic test indicate the increase of phagocytosis in the organism immune to tularemia, but these data are only of relative significance, because the main force of the organism counteracting the pathogen lies in the cellular elements of the fixed connective tissue rather than in the blood leukocytes, which was established by V. K. Vysokovich. The leading significance of phagocytosis as a factor in the protection of the immune organism against reinfection has been proved in plague (M. P. Pokrovskaya and L. S. Kaganova), brucellosis (P. A. Vershilova and I. N. Kokorin), rickettsial diseases (I. N. Kokorin) and there is no reason for the belief that in this respect tularemia constitutes an exception. According to the data of I. N. Kokorin (1956), the morphologic criterion of the immunological reorganization in brucellosis and rickettsial diseases should be considered the macrophagic reaction and increase in the processes of cell metabolism (a directed change in the synthetic processes, the appearance of qualitatively different cells rich in ribonucleic acid and polysaccharides). The increase in the digestive power of the macrophages finds its expression in the complete phagocytosis even of microorganisms which are difficult to lyse and intracellular digestion of them in the first 48-72 hours after infection. This macrophage activity is associated with their production of protective substances (antitoxins and lysins) which act specifically on the given microbe, which makes it possible for macrophages to detoxify it and assimilate it by means of intracellular digestion. Subsequent reorganization of polymorphonuclear leukocytes has not been noted. Aside from the inflammatory reaction and phagocytosis we should keep in mind other possible methods of protecting the organism immune to tularemia against reinfection, particularly the accumulation of antifibrinolysins in the blood serum (Ye. N. Aleshina and T. I. Puchkova, 1947).

In concluding the presentation of the section on mechanisms of immunity in tularemia it should be noted that they are approximately the same as in a number of infectious diseases of similar pathogenesis -- brucellosis, plague, tuberculosis, rickettsial diseases -- and the existing data fit within general concepts of mechanisms of protection against bacterial infections (Sh. D. Moshkovskiy, 1947; L. A. Zil'ber, 1948, 1959; P. F. Zdrodovskiy, 1950, and others).

### Conclusion

The most important results in the immunology of tularemia have been obtained as the result of a comparative study of antigenic substances of virulent, slightly virulent (vaccine) and avirulent strains of tularemia bacteria and detection of the effect of the carriers of these antigens on the animal organism with different infectious sensitivities and immunizabilities. The comparative method of investigation used made it possible to determine the antigenic complexes of the microbe with which its virulence and protective properties are associated and to demonstrate a number of the general rules and regulations of creation of immunity against tularemia and some of its characteristics in various species of animals and in man.

A particularly important achievement in the field of immunology of tularemia should be considered the living vaccine worked out by B. Ya. El'bert and N. A. Gayskiy. With respect to the duration of immunity conferred on man and the degree of its expression the tularemia vaccine is not inferior to smallpox vaccine, which to date is considered the best of all the known vaccines in world practice. The use of tularemia vaccine experimentally has markedly expanded the possibilities of comparative study of immunity in tularemia, enriching the immunology of this infectious disease with new facts. The use of the vaccine in practice has led to a marked reduction in the incidence of tularemia in people and, in places, to an almost complete elimination of it.

Very detailed investigations on the immunological reactions and the establishment of their connection with immunity in tularemia should be considered an important achievement. Data existing on this subject constitute the scientific basis for the laboratory diagnosis of tularemia as well as for the determination of the duration of immunity in those inoculated or in those who have had tularemia. Investigations on passive immunity have not given any essential practical results for therapy of tularemia, just as is the case in many other bacterial infectious diseases. However, in the general line of investigations on the immunology of tularemia the data obtained in this section are of defin-

ite significance and should be given appropriate consideration.

The greatest number of gaps is being felt in the development of problems of the mechanisms of immunity in tularemia. The entire section of phagocytosis needs special study; additional studies are needed on antibodies, nervous and humoral regulation of immunogenesis, etc. Special attention should also be given to problems of the creation of immunity in accordance with age and other characteristics of the organism, and then in mixed infectious diseases, associated vaccination, the effect of various external agents including ionizing radiation, etc. It is to be hoped that in the near future these gaps will be filled in by research workers.



## Chapter X

### Vaccinoprophylaxis of Tularemia

#### General Comments

Work has been done on the development of a vaccine against tularemia in the USSR, United States and other countries. However, only Soviet scientists have succeeded in solving this problem of very great importance for public health practice with complete success. The theoretical aspects of this problem as well as experimental basis for the use of various vaccines have been presented in Chapter IX. In the present Chapter we are analyzing the data on vaccination of people including the organization of inoculations and evaluation of their effectiveness.

In 1931 L. M. Khatenever and G. Ya. Sinay in Southeast Kazakhstan (the village of Ush-Tobe) inoculated 41 persons with a tularemia glycerinated vaccine killed by heat (L. M. Khatenever and L. A. Levchenko, 1935). This was the first experiment in vaccination of people against tularemia in the world. The inoculations were given subcutaneously, once, twice, and three times; in the last two cases with intervals of five days between the inoculations. It was determined that after triple vaccination in the majority of persons inoculated agglutinins appeared in the blood serum; those inoculated were under observation a total of only three weeks, and the prophylactic effectiveness of the vaccination remained unclarified. Subsequently, killed corpuscular tularemia vaccines were tested repeatedly on people, but the prophylactic effect of the inoculations was either low or remained unclarified (Ye. M. Tsvetkova, 1944). There is only a single statement concerning a good result in preventing tularemia in people inoculated subcutaneously with killed glycerinated vaccine shortly before the occurrence of an arthropod-borne outbreak, whereby only those who were not inoculated became sick (L. M. Khatenever, 1946). Attempts at vaccinating people with killed tularemia vaccines were made in the United States also, but they were of a localized character, whereby the inoculations protected poorly against tularemia (see Chapter II).

B. Ya. El'bert and N. A. Gayskiy believed that only living tularemia vaccine can provide a persistent and strong immunity in the inoculees. As has been pointed out in Chapter IX, they worked out the theoretical basis for specific prophylaxis of tularemia which subsequently found practical confirmation in the preparation and application of tularemia vaccine made of living attenuated microbes by M. M. ...

Faybich. The first test of an attenuated tularemia strain ("Moskva") was made on 10 volunteers by B. Ya. El'bert and N. A. Gayskiy, and it showed the harmlessness of this strain for people and its high degree of immunogenicity (judging by the immunological reactions). Testing of attenuated tularemia cultures on people was renewed by N. A. Gayskiy in 1942 after he obtained attenuated strains with the necessary residual virulence by the laboratory method. Fifty workers of the Irkutsk Plague-Control Institute expressed the desire to be given an experimental subcutaneous vaccination, and six of them asked for subsequent experimental infection with a virulent culture of the tularemia microbe. N. A. Gayskiy mentions the names of the first participants of this test: N. D. Altareva, A. V. Korotkova, Ye. P. Makarova, T. G. Linnik, A. G. Lopotukhina, V. Ya. Mikhaleva, V. N. Rychkova and others. This test, conducted under the clinical observation of V. V. Kosmachevskiy (1944), confirmed the harmlessness of the vaccine for people and its good protective properties, because none of the inoculated infected half-year after vaccination with a virulent culture of *B. tularensis* became sick with tularemia.

As early as the end of 1942 the vaccine began to be used in foci of tularemia. In December 1942 under the personal supervision of N. A. Gayskiy 1300 persons were inoculated subcutaneously in Kirlovskaya Oblast; in 1943, 2,214 in Voronezhskaya Oblast; and about 200 persons in Kazakhstan. The vaccine possessed good protective properties but also had essential defects; it was prepared in the form of a suspension in physiological saline solution and after standing it rapidly lost its immunogenic properties (see below) and also produced excessive side-effects. With the aim of prolonging the longevity of the vaccine N. A. Gayskiy and Ye. M. Golinevich as well as M. M. Faybich and T. S. Tamarina used vacuum drying of the vaccine in 1944, whereby the latter authors brought the development of this problem to its practical completion. M. M. Faybich and T. S. Tamarina (1946) prepared a vaccine first for subcutaneous and then for percutaneous methods of vaccination. This vaccine was called "dry living tularemia vaccine of the NIIEG". It possessed considerable advantages over the liquid vaccine in the fact that it could be preserved at a temperature of 0-2° up to two years or more without losing its immunogenic properties. The method of preparing the vaccine made it possible accurately to dosage the number of microbes in the preparation, which could not be done in the preparation of liquid vaccines. These valuable properties of the dry tularemia vaccine contributed to the fact that at the present time it is considered the best of the tularemia vaccines which have been proposed, and in recent years only the dry vaccine has been used (of the NIIEG type) in the USSR.

In 1945, B. Ya. El'bert along with I. S. Tinker, T. I. Puchkova and others (1945, 1946, 1947) worked out and suggested a percutaneous method of vaccination against tularemia and prepared a special liquid living vaccine (from the Gayskiy 15 strain) on M. S. Drozhevskina's yolk medium. B. Ya. El'bert's vaccine had an advantage over the liquid vaccine of N. A. Gayskiy in that when it was kept properly it did not lose its immunogenic properties for two or three months. However, the main and most valuable element of B. Ya. El'bert's suggestion was the percutaneous method of vaccination, which reduced its side-effects, simplified the technique of giving the inoculation and made it possible to conduct the observation of the "take" of the vaccine. At the end of 1945, B. Ya. El'bert, I. S. Tinker, T. I. Puchkova and others (1947) vaccinated a considerable number of people in foci of tularemia and obtained good results; 12 days after conducting the vaccination cases of tularemia were no longer observed among the inoculees.

The use of inoculations by the percutaneous method in anti-epidemic practice represented a new stage in the vaccination of people against tularemia. This method was exceedingly valuable in the practical prophylactic work of medical workers. Practice has shown that the Soviet scientists N. A. Gayskiy, B. Ya. El'bert and M. M. Faybich succeeded completely in solving the problem of the specific prophylaxis of tularemia and in preparing highly effective living tularemia vaccines which reliably protect people against disease, vaccines the likes of which do not yet exist abroad. In the presentation below we shall deal only with the data of study of living vaccine. At the present time, the bulk of it is still prepared from the Gayskiy 15 (reconstituted strain).

#### Properties of Living Tularemia Vaccines

**Liquid Gayskiy Vaccine (vaccine virus).** The liquid living Gayskiy virus vaccine was prepared from a natural suspension of the culture by means of dilution with sterile physiological saline solution to a standard of 50,000,000 microbes per cc. The vaccine was injected subcutaneously, once, in a dose of 0.4-0.5 cc (20,000,000-25,000,000 microbes). For children Gayskiy prepared another vaccine containing 10,000,000 microbes per cc. At ages of 10-16 it is recommended that 0.5 to one cc of this (children's) vaccine be injected, or 5,000,000-10,000,000 microbes. The vaccination created a strong immunity for a long time in the inoculees. According to the data of N. A. Kazbaryuk (1949), in vaccination with the Gayskiy virus vaccine the allergic skin reactivity was preserved up to three years (the observation

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period) in 82 percent of the inoculees. Through the subsequent observations of V. S. Sil'chenko (1952) on these inoculated persons it was found that after six years the allergic skin reactivity amounted to 70.6 percent. A considerable number of the inoculees lived in an area of existing natural foci of tularemia and did not become sick, while tularemia did occur among the persons who were not inoculated.

The main defect of the vaccine was short longevity (possibilities of preservation), because the microbes rapidly died out in it, which led to a gradual reduction of the immunogenic properties up to the point of complete loss of them. N. D. Altareva (1946) determined the fact that after keeping the mother suspension in a refrigerator for 40 days its "take" was reduced by 23 percent, and after preservation under room temperature conditions, by 78 percent. As a result of this it was necessary to prepare the vaccine from the mother suspension on the spot or else ship it in thermos bottles containing a packing of ice with salt. This property of the vaccine created considerable difficulties in the organization of inoculations in foci of tularemia. In the first experimental inoculations V. V. Kosmachevskiy (1944) noted post-inoculation side-reactions in a considerable number of those vaccinated. Thus, of 50 persons inoculated a general and local associated reaction occurred in 36 (72 percent); this included 23 in whom a temperature elevation from 37 to 38° was observed and three in which it went up to 39°; in 17 persons there was an enlargement of the lymph nodes to the size of a bean and the size of a walnut. These data were noted in the foreign literature (Foshay, 1950; Juszatz, 1952) as an example of excessive side-effects of the vaccine, although V. V. Kosmachevskiy considered this vaccine harmless to the inoculees in the work which he published.

Liquid Yolk Vaccine of El'bert. In view of the fact that experimentally the possibility of prolonged preservation of living tularemia microbes was established in liquid yolk medium (up to one year), B. Ya. El'bert utilized this medium (10 percent egg yolk suspension in physiological saline solution) for the preparation of a tularemia vaccine; this had been proposed by M. S. Drozhevskina. A culture of the vaccine strain was made in liquid yolk medium; cultivation assured a high bacterial concentration. The method of production was simple, but it did not afford the possibility of accurately dosaging the number of microbes in a certain volume of the preparation. For the purpose of producing the liquid yolk vaccine only the Gayskiy 15 strain was used.

The longevity of the liquid yolk vaccine was first found to be three months, but in practice it was shown that in the summertime, particularly when kept at room temperature, the vaccine lost its im-

munogenic properties much before that and the success of inoculation with it was markedly reduced. This led to the establishment of a new maximum longevity of the vaccine -- two months -- when kept at a temperature of 4 to 10°. The inoculations were given once. On the skin of the arm (in the upper third) three-four superficial linear scratches were made at a distance of one-two centimeters from one another, and a drop of the vaccine was rubbed into them. B. Ya. El'bert made it clear that in the inoculees, on the fourth-sixth day at the site of injection of the vaccine, specific reactive skin phenomena appeared (see below) which made it possible to judge the success of the vaccination. In those inoculees in whom the vaccine "took" a skin allergic reactivity developed (as determined by the tularin test), and somewhat later agglutinins were found in their sera. In the inoculees a strong immunity was elaborated by the seventh-14th day, which was confirmed by a considerable reduction of the incidence of disease among the inoculees during the first 10 days and a complete elimination of cases of disease among the inoculees 12-14 days after vaccination. These data were established by B. Ya. El'bert and coauthors (1947) on a large number of inoculees during a great outbreak of tularemia in 1945. In view of the impossibility of attaining an exact concentration of microbes in the liquid yolk vaccine different series of it gave different degrees of side-reactions in the inoculees.

During the first few years of incorporation of tularemia living vaccines into practice the inoculations were basically conducted with liquid yolk vaccine of B. Ya. El'bert. Only beginning with 1950-1951 were public health organs able to proceed with the use of dry tularemia vaccine.

Dry Living Tularemia Faybich Vaccine (NIEG). M. M. Faybich and T. S. Tamarina (1946) used a method of drying a frozen suspension of vaccine culture in a special drying and growth medium in a high degree of vacuum for the preparation of dry tularemia vaccine. The work was conducted according to the method suggested by M. M. Faybich in an apparatus worked out by NIEG workers R. V. Karneyev, B. S. Del'nik and others. First, for the preparation of M. M. Faybich vaccine, attenuated Gayskiy 15 strain was used; later, strains 10 and 33 were used obtained at the NIEG by M. M. Faybich and coauthors. From the last two strains vaccine was prepared in a mixture with the 15 strain.

The advantage of dry tularemia NIEG vaccine over liquid Gayskiy virus vaccine and El'bert's egg yolk vaccine lay in the fact that in the former it was possible to measure out a relatively constant number of living microbes in the preparation and preserve the vaccine for a long time after it was prepared. Study of the properties of the

dry vaccine showed that drying of cultures of the vaccine strain leads to prolonged preservation of living microbes in the preparation, whereby the biological titer of the suspension does not change. According to the data of M. M. Faybich and T. S. Tamarina (1946) the biological titer of the vaccine, equal to  $10^{-8}$  and  $10^{-9}$  before drying did not change either after drying or a year after keeping the vaccine at a temperature of  $2-4^{\circ}$  (in vacuum-sealed ampules). (For the purpose of determining the biological titer of the vaccine it was diluted by 10, 100, etc. times, and from every dilution a culture was made in test-tubes with coagulated egg yolk medium; the titer was established by the least dilution which showed growth of the culture. At the present time, a better method for determining the number of living bacteria in the vaccine is used -- minimum dilutions are plated out on petri dishes containing Yemel'yanova medium (see Chapter III), and the number of colonies which grow out are counted. Simultaneously, consideration is given to the number of SR and R colonies, that is, of the immunogenic O (Vi) and non-immunogenic O types. This permits a very accurate evaluation of the quality of the vaccine). Keeping the vaccine at a higher temperature led to a reduction in the number of living microbes: at a temperature of  $18^{\circ}$  the biological titer after 350 days dropped to  $10^{-4}$  and  $10^{-5}$ , and at a temperature of  $26^{\circ}$  the same reduction in the biological titer was observed after 75-90 days.

A. D. Zlatkovskiy used a dry vaccine for the vaccination a year after its preparation (with observance of a routine of keeping it) and in all the inoculees he observed the appearance of allergic skin reactivity attesting to the "take" of the vaccine and the occurrence of immunity in the inoculees. In this way it was established that dry tularemia NIEG vaccine when kept in the cold preserves its immunogenic properties for two years. The vaccine can be kept at room temperature also (from  $18$  to  $20^{\circ}$ ), but its longevity is reduced to 300 days. Considering the fact that a temperature routine is not maintained everywhere in keeping the vaccine, at the present time a one-year longevity period for the vaccine has been established (the maximum permissible time it can be kept before using it) under conditions of preservation at a temperature of  $4$  to  $10^{\circ}$ .

After experiments on animals M. M. Faybich proceeded with the study of the effect of dry vaccine on people. Fifty-two volunteers were vaccinated; the vaccine was given to them subcutaneously in doses of 7,500,000 and 250,000,000 microbes. Then, more than 30,000 persons were inoculated who were given from 12,500,000 to 25,000,000 microbes (according to the GKI standard). The observations showed that doses of vaccine containing up to 50,000,000 microbes are well tolerated and produce a slight local reaction and in rare cases

a febrile and slight regional lymph node reactions. The doses which do not exceed 25,000,000 microbes, even in those who have had the disease, do not produce any marked allergic reaction. On the basis of these observations it was determined that the optimum dose (producing a good immunizatory effect and not causing any pronounced side-effects) for people after subcutaneous injection is 12,500,000-25,000,000 microbes. Subsequently, it was determined that for percutaneous immunization the vaccine should contain 2,000,000,000 microbes per cc. Recently, as the result of the incorporation of strains with a partially increased activity into vaccine production (the Gayskiy 15 reconstituted strain and the Yemel'yanova 155) as well as because of an improvement in the technology of preparing the vaccine and methods of testing it this standard has been reduced to 1,000,000,000 microbes per cc (O. S. Yemel'yanova, 1957).

First, the dry NIEG vaccine was prepared for subcutaneous injection. Before vaccination the contents of the ampule (the dry microbe mass) were diluted with sterile physiological saline solution (in the volume indicated on the label of the ampule). The dose of the diluted preparation injected was 0.5-one cc, which amounts to 12,500,000, 25,000,000 microbes according to the GKI standard. Beginning with 1946 the NIEG began to put out vaccine for percutaneous use. In this method it was recommended that two or three drops of the vaccine be applied to the skin of the middle third of the arm, and through these drops two or three parallel scratches be made. In the case of mass inoculations the dry NIEG vaccine assured a higher "take" (see below) after percutaneous use but also caused side-effects in a somewhat larger number of inoculees than did the liquid yolk vaccine (particularly since it was applied to the skin in a larger quantity than the yolk vaccine).

#### "Take" of the Vaccine

A study of the properties of living tularemia vaccine, both liquid and dry, showed that they "took" in a high percentage of cases. B. Ya. El'bert (1946), using liquid yolk vaccine shortly after its preparation, noted 98-99 percent success. A. V. Mashkov (1947), using the vaccine two days after it was produced, obtained 99 percent success. In other materials published the most varied data are presented on the degree of "take" of the liquid vaccine. Yu. A. Myasnikov, V. S. Sil'chenko, M. I. Tsareva, M. F. Shmuter, V. A. Yudenich and others noted success of vaccination in 85-96 percent of the inoculees; Ye. A. Dem'yanov, in 63 percent; G. P. Uglovoy, I. N. Mayskiy and others, in one of the regions observed an exceptionally low "take" --

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from zero to 52 percent. According to the summarized data of N. G. Olsuf'yev (1953) the success of the liquid yolk vaccine amounted to 85.1 percent on the average. As was made clear, various cases of poor success with the liquid yolk vaccine depended on the fact that it had been kept in the warm, on the utilization of vaccine series in which the longevity had been exceeded, violations of the inoculation technique, particularly treatment of the skin with strong-acting disinfectants (chloramine, iodine and others), etc. (V. S. Sil'chenko, 1953).

With the use of the dry NIEG tularemia vaccine more uniform indices of good success were obtained with it, attesting to the stability of the preparation, better preservation of immunogenic properties under the same preservation conditions as the liquid vaccine. N. G. Olsuf'yev (1953), after generalizing on a large number of observations of different authors, pointed out that the success of dry tularemia vaccine amounts to 97.9 percent, on the average, varying within limits of 93.5-100 percent. Even these indices for the dry vaccine are evidence of its superiority over the liquid yolk vaccine. In 1954-1955 a certain reduction in the success of the dry vaccine was noted, which N. G. Olsuf'yev and coauthors (1958) tied in with a reduction in the residual virulence of the Gayskiy 15 vaccine strain. After the valuable properties of the Gayskiy 15 vaccine strain had been restored in the tularemia laboratory of the IEM imeni Gamaleya and the new 155 Yamel'yanova strain had been incorporated into production and there had been an improvement in the technique of vaccine production the success of inoculation with this preparation reached 98-100 percent (Table 28).

B. Ya. El'bert pointed out that in the percutaneous method of vaccination the success of inoculations with the vaccine is readily determined by the development of a post-inoculation reaction on the skin (at the site of application of the vaccine) consisting of the appearance of reddening of the skin, swelling and small vesicles which were then covered by crusts in the areas of scarification. Further observations by V. S. Sil'chenko (1948, 1953), G. P. Uglovoy and coauthors (1953), V. A. Yudenich (1953) and others confirmed these data. These authors in cases of successful vaccination observed the successive development of local skin post-inoculation phenomena. During the first two or three days on the skin there were traces of scratches made during vaccination. On the fourth-sixth day (less often, somewhat later, up to the eighth-10th day) the skin at the inoculation site became red and swollen. At the site of the scratches small raised margins were formed which were elevated above the skin surrounding them (Fig 70). The areas of swelling and hyperemia were of different sizes -- from 0.5 to one-two centimeters. During this period in the majority of



Table 28

## Success of Inoculation with Tularemia Vaccines (Liquid and Dry) in the Vaccination of People

1 Тип вакцины	2 Автор	3 Год наблюдения	4 Число принятых	5 Успешность, %
6 Жидкая желточная	12 В. С. Сильченко,	1946	93 516	89,6
7 То же	13 А. А. Казберук	1948—1951	23 972	89,5—94
" "	14 А. А. Демьянов	1948	2 015	63,0
" "	15 Н. Г. Олсуфьев (сводные данные)	1946—1950	17 500	85,1
8 Сухая НИИЭГ	16 В. С. Сильченко	1947	12 444	93,5
7 То же	17 Г. П. Угловой	1948	383	97,3
" "	18 Н. Г. Олсуфьев (сводные данные)	1946—1950	3 200	97,9
9 Сухая ИЭМ им. Гамалеи	17 Г. П. Угловой	1953	453	98,6
7 То же	18 А. П. Кульба	1954	282	84,3
" "	19 Л. С. Матвеев	1955	59	86,4
10 Сухая ИЭМ им. Гамалеи (из штамма Гайского 15-восстановленного)	19 Л. С. Матвеев	1955	205	100,0
7 То же	20 Н. Г. Олсуфьев			
" "	21 С. С. Арофьев			
" "	22 В. С. Сильченко и др.	1953	1 678	98,5
" "	23 Н. Г. Олсуфьев			
" "	24 О. С. Емельянова			
" "	25 В. С. Сильченко, Ю. А. Мисников и др.	1958	6 716	97,9
11 Сухая ИЭМ им. Гамалеи (из штамма 155)	19 Л. С. Матвеев	1955	249	100,0
7 То же	23 Н. Г. Олсуфьев			
" "	24 О. С. Емельянова, В. С. Сильченко, Ю. А. Мисников и др.	1958	6 600	98,1

1. Type of vaccine; 2. Author; 3. Year of observation; 4. Number of inoculees; 5. Success of inoculation, %; 6. Liquid yolk; 7. Same; 8. Dry NIEG; 9. Dry IEM imeni Gamaleya; 10. Dry IEM imeni Gamaleya (from the Gayskiy 15 reconstituted strain); 11. Dry IEM imeni Gamaleya (from the 155 strain); 12. V. S. Sil'chenko; 13. N. A. Kazberuk; (legend continued next page)

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[legend continued from previous page] 14. Ye. A. Dem'yanov; 15. N. G. Olsuf'yev (composite data); 16. V. S. Sil'chenko; 17. G. P. Uglovoy; 18. A. P. Kul'ba; 19. L. S. Matveyets; 20. N. G. Olsuf'yev; 21. S. S. Aref'yev; 22. V. S. Sil'chenko and others; 23. N. G. Olsuf'yev, O. S. Yemel'yanova, V. S. Sil'chenko, Yu. A. Myasnikov and others; 24. More than.

inoculees a sensation of itching of the skin in the area where the vaccine reaction develops occurred. In the sixth-10th day hyperemia and swelling are intensified; the area of them is expanded. At the tops of the raised margins small vesicles can appear -- vesicles the size of a pinhead to the size of a grain of millet. On the 10th-15th day the vesicles become confluent and are converted into pustules (in some cases the development of vesicles occurs without a transition into pustules). On the 12th-19th day phenomena of inflammation (redness, swelling) are notably reduced. The vesicles and pustules begin to be covered with scabs. At the site of the former large confluent vesicles an eschar is formed (Fig 71). At this time, at the site of the pustules a small infiltrate is found which is gradually resorbed. The scabs remain for five-10 days, and then fall off, and a small whitish scar or spot (depigmented) remains in place of them on the skin. In a number of inoculees the scabs remain somewhat longer, particularly when an eschar is formed (until the 30th-40th day after the time of inoculation).

The entire developmental cycle of the post-inoculation skin phenomena lasts from 20 to 40 days. The degree of expression and the duration of these reactions depend on the degree of reactivity of the body and the skin characteristics of the inoculees. Increased skin sensitivity can also be explained by the fact that in some inoculees, on the sixth-15th day individual vesicles appear on the skin at some distance from the scratches but in the area into which the vaccine has been rubbed. I. L. Martinevskiy (1953) observed the quite frequent occurrence of such additional reactions (in 20.7 percent) in the inoculees; they were located at a distance of 0.2-nine centimeters from the scratches. The author relates their occurrence to rubbing in and smearing of the vaccine with the clothing.

The times that we have given for the development of the post-inoculation skin reactions in part of the inoculees show deviations in the direction of a reduction or lengthening (see below). It must be supposed that in addition to the reactive characteristics of the bodies of the persons vaccinated overdosage of the vaccine may be of significance here (acceleration and greater degree of expression of the reac-



**Fig 70. Skin Reaction on the Fifth Day after Vaccination (Photograph by G. F. Uglovoy).**



**Fig 71. Formation of Scabs at the Site of Skin Inoculation on the 15th Day after Vaccination (Photograph by G. P. Uglovoy).**

tion) or the use of a vaccine with a reduced number of living bacteria as a result of their partial extinction after long and incorrect preservation of the preparation (later occurrence and weaker expression of the reactions). B. Ya. El'bert (1945-1946) considers the skin response to the injection of vaccine the main index of the vaccine process and, therefore, of the success of immunization. Other authors also make the skin inoculation reaction the basis for evaluation of success of vaccination.

In the majority of the inoculees shortly after vaccination

the occurrence of pronounced skin inoculation reactions occurs which is evidence of the fact that the vaccine has "taken". In a certain number of the inoculees these changes are poorly expressed and appear later. Against the background of slight hyperemia and slight swelling in the area of scratches the individual small vesicles can be discerned with difficulty. However, as the investigations showed, the distinctness of the skin reaction depends not only on the quality and quantity of vaccine applied to the skin but also on the reactivity of the bodies of the inoculees. In various inoculees and in the presence of slight skin reactive phenomena a quite strong and stable immunity can be created. In doubtful cases the best method of checking on the success of the vaccination is the performance of the percutaneous or intradermal test with tularin. The presence of an allergic reaction in the subjects thereby is evidence of an immunological reorganization of the body (as the result of vaccination or of having had tularemia). The observations showed that in all people in whom skin inoculation reactions were observed allergic reactivity of the body also occurs and, therefore, the test with tularin (percutaneous or intradermal) will give a positive result.

The time of occurrence and development of the post-inoculation skin phenomena, according to the data of the majority of authors, is the same. B. Ya. El'bert (1946), as a rule, noted that these reactions occurred on the fourth day after inoculation and obtained their maximum degree of expression on the fifth-sixth day. Yu. A. Myasnikov, V. S. Sil'chenko, M. I. Tsareva, V. A. Yudenich (1953) also noted the development of skin reactions in the majority of inoculees beginning with the fourth-fifth day, but simultaneously they observed their later occurrence in part of the subjects: on the sixth-eighth day. In eight-nine percent they even occurred on the 10th-11th day. Extremely rarely these reactions occurred later (on the 20th day or later), which has been reported by M. F. Shmutor (1953). In the past these data constituted the basis for recommending a check on the success of vaccination in the period between the 12th-15th day and a second check on the 20th-22nd day in those inoculees in whom the skin vaccination reaction was absent or indistinct at the time of the first examination. At the same time, for the purpose of checking the success of the vaccine in revaccination, considering the occurrence of an allergic reaction in the inoculees (with the presence of immunity) in the early period -- on the second-third day (see "revaccination" section), a check of those revaccinated was recommended on the second-fifth day. Therefore, in conducting the inoculations in an area of natural foci and in places where inoculations were conducted previously the inoculees had to be checked up to three times -- on the second-fifth, 12th-15th, and

20th-22nd days.

After O. S. Yemel'yanova (1957) succeeded in restoring the immunogenic properties to the Gayskiy 15 vaccine strain and obtaining the new 155 vaccine strain the tularemia vaccine prepared from these strains began to give clear-cut skin reactions after percutaneous application in the great majority of persons vaccinated, as early as on the fifth-eighth day, and in persons who were revaccinated, after two days (Table 29).

Table 29

Time of Appearance of Skin Inoculation Reaction in Those Vaccinated and Revaccinated with the Vaccine Prepared from the Reconstituted Gayskiy 15 and Yemel'yanova 155 Strains

Группы ①	Всего ②	% лиц с кожной прививочной реакцией через			
		2 суток ④	5 суток ⑤	8-9 суток ⑥	12-15 суток ⑦
③ Инфицированные . . .	13 322	—	82,2	96,1	98,0
③ Ревакцинированные . . .	5 401	100,0	99,9	89,9	78,9

According to the data of a mass check made in 1958 by the tularemia laboratory of the IEM imeni Gamaleya in conjunction with peripheral institutions. 1. Groups; 2. Total; 3. % of persons with skin inoculation reaction after: 4. Days; 5. Vaccinated persons; 6. Revaccinated persons.

The decision was made to conduct a single check of the inoculees (both vaccinated and revaccinated) in the period between the fifth and seventh days. A second check between the 12th and 15th days was given only to those vaccinated persons (approximately seven-10 percent) in whom there were negative or doubtful results at the time of the first check.

#### Vaccination Technique

Dry tularemia NIEG vaccine was prepared both for percutaneous and subcutaneous methods of vaccination. Recently, the inoculations have been given exclusively percutaneously; therefore, we

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shall discuss the technique of vaccination by this method only. The technique is extremely simple and similar to vaccination against smallpox. The dry vaccine is put out in glass sealed ampules, and a solvent (distilled water) prepared in a sterile manner is appended to it (in separate ampules).

Directly before beginning the inoculations the vaccine is diluted; for this purpose the neck of the ampule containing the vaccine is nicked with a file, rubbed in alcohol and cautiously flamed over a burned so that the ampule contents (dry vaccine) are not heated. In the same way the ampule is also treated with the solvent. Then, the necks of both ampules are broken off, and distilled water is transferred to the ampule containing dry vaccine by means of a sterile syringe in a quantity indicated on the label of the ampule; the ampule containing these contents is shaken until a homogeneous suspension is obtained, and the vaccine is ready for use.

The inoculation is usually given on the outer surface of the middle third of the left arm. At the site of the inoculation the skin is first cleaned with alcohol and then disinfected with ether. However, in practical work the skin is usually treated with alcohol alone. When alcohol or ether evaporates a single drop of vaccine is applied with an eyedropper in each of two places at a distance of three-four centimeters from each other (a total of two drops). Then, in each area of skin on which the drop of vaccine has been applied two parallel scratches 0.8-one centimeter in length each are made with a vaccination quill. In all, therefore, four scratches are made and two drops of vaccine are used per person. The scratches should be made superficially but not so shallow (and short) as is now done for smallpox vaccination; the scratches should be applied until small droplets of blood appear (but not excessive bleeding). Scratches more than one centimeter in length should not be made, because this can lead to excessive reactions. Then, with a glass rod or the flat side of the vaccination quill the vaccine is rubbed into the scratches on the skin for a half-minute. Care should be taken thereby that the vaccine does not go beyond the limits of the scratches and more should be concentrated in the area of them. After this, the vaccinated person is kept for five-10 minutes (until the vaccine dries completely) with his arm exposed, and this concludes the inoculation.

In view of the fact that after dilution with physiological saline solution or other solvent the dry vaccine acquires a liquid consistency and a drop of it applied to the skin is rapidly diffused, some authors recommend applying the drop of vaccine after the scratches have been made, rather than before, directly in the area of the scratches. According to the data of various authors both methods are equivalent.

lent and can be used in the practice of vaccination.

In the vaccination of pre-school age children (two-six years) they are given only one drop of the vaccine and a total of two scratches no more than 0.5 centimeter in length each is made. It is categorically forbidden to inject the vaccine for percutaneous application subcutaneously. For the purpose of drying the skin before the inoculation it is forbidden to use strong-acting disinfectants (chloramine, carbolic acid, iodine and others), because they kill the living microbes of the vaccine, which leads to a reduction in the effectiveness of the inoculations. As has been mentioned, alcohol or ether are used for the disinfection or else a mixture of alcohol and ether (50 cc of each substance). The alcohol should be allowed to evaporate until the skin surface is dry; otherwise, the effectiveness of the inoculation can be reduced. In giving the inoculations it is necessary to be guided strictly by the printed instructions inserted into boxes containing the vaccine.

The technique of percutaneous vaccination is so simple that every well-instructed medical worker can give the vaccinations successfully by himself.

#### Reactive Phenomena Associated with Vaccination

At the time of the first check on the Gayskiy virus vaccine V. V. Kosmachevskiy (1944) noted side-effects in the inoculees which occurred after subcutaneous injection of tularemia vaccine. A temperature elevation occurred in half of those inoculated; an enlargement of the lymph nodes, in one-third; tenderness in the liver, in one-third; but all these phenomena rapidly disappeared without trace.

Further studies of the side-effects produced by living tularemia vaccines showed that in part of those inoculated with both liquid and dry vaccine there are associated reactions. However, in the published materials there is no agreement as to the frequency with which these reactions occur and their degree of expression. B. Ya. El'bert (1946) observed slight associated reactions in eight-10 percent after percutaneous inoculations with liquid egg yolk vaccine: a slight temperature elevation (to 37.2-37.8°) and a very transitory pain in the axillary region. N. G. Olsuf'yev and coauthors (1950) pointed out that side-reactions to the inoculation, as a rule, are moderate and do not cause any loss of the ability to work in those inoculated.

Associated reactive phenomena can be divided into the following three groups: a) general subjective: a feeling of malaise, depression, weakness, chill, headaches, nausea, etc.; b) general objective: temperature elevation; c) local: pain in the axillary region and enlargement of the axillary (regional to the site of inoculation)

lymph nodes.

The frequency with which these reactions occur in inoculees is different according to the data of different authors (Table 30).

According to the data in Table 30 it is seen that in the inoculees there is a predominance of subjective phenomena which do not trouble them very much. In second place is enlargement of the lymph nodes and finally, temperature elevation. Associated reactive phenomena (with the exception of lymphadenitis) usually occur on the second-third day after the inoculation and much less often, on the fourth-fifth day. They last one-two days and disappear completely without trace. The temperature rises to  $37.2-37.6^{\circ}$ , and in rare cases (in individual inoculees), to  $38^{\circ}$ . Subjective phenomena, as a rule, are slight; the inoculees do not usually complain of them, and the physician learns about them only after detailed questioning. This once again is evidence of the low degree of expression of associated reactive phenomena. No loss of the ability to work as the result of the pronounced reactive phenomena was observed by the majority of investigators or else it occurred only in occasional inoculees (one-two persons per 2,000-3,000 inoculated), whereby it lasted one or two days. Only G. P. Uglovoy (1953) in one group of inoculees observed a brief loss of the ability to work in 2.4-4.5 percent of the inoculees, but he believes that people who in the past had had tularemia might have been in the group of those vaccinated persons who showed pronounced side-effects. This author indicates the considerable frequency and degree of expression of side-reactions in those who have had tularemia given vaccination, which was confirmed by the observations of V. A. Yudenich (1954).

Local associated reactions consist of the occurrence of pain in part of the inoculees; in another part, enlargement of the regional (axillary) lymph nodes. The pain usually appears on the third-seventh day, while the formation of axillary lymphadenitis occurs on the seventh-20th day. In view of the fact that the vaccine, as a rule, is applied to the left arm, lymphadenitis usually occurs in the left axillary fossa; where the vaccination was given in the right arm the occurrence of right-sided lymphadenitis was noted. A feeling of pain (in the absence of lymphadenitis) disappears without trace after three-five days. The size of the vaccine lymphadenitis varies from the size of a pea to the size of a bean or cherry and very rarely larger. In some of the inoculees the lymph nodes, after reaching a certain size in the first few days, did not enlarge further; in others, enlargement of the lymph nodes continued for two-four weeks. Post-inoculation lymphadenitis during the five-45 days after they appear undergoes a gradual complete resorption and disappears without trace. Only in a small number of inoculees does the complete resolution of the lymphad-



Table 30

Side-Effect Production of Tularemia Vaccines in the Vaccination of People against Tularemia

1 Тип вакцины	2 Автор	3 Год наблю- дения	4 Число при- внутых	5 % лиц с сопутствующими реакциями				
				всего	6 в том числе			
					7 увели- чение лимфа- тиче- ских узлов	8 субъ- ективные жалоб- ы	9 повы- шение темпе- ратуры тела	10 утрата трудоспо- собности
11 Вирус-вакцина Гайского	В. В. Космачев- ский	1942	50	72,0	34,0	32,0	52,0	4,0
12 Жидкая желточная вакцина	В. С. Сильченко	1946	93 516	23,3	4,5	13,6	4,8	—
13 То же	Н. А. Казбрюк	1948	23 972	24,6	3,6	12,8	8,2	—
14 Сухая НИИЭГ	В. С. Сильченко	1947	12 444	32	9,2	15,0	7,5	0,3
15 То же	Г. П. Угловой	1948	383	24,3	6,5	16,6	2,1	3,6
16 Сухая Смолен- ского ИЭМ	В. А. Юдонич	1952	15 862	16,1	2,2	8,7	5,2	—
17 Сухая ИЭМ им. Гамалеи	Г. П. Угловой	1953	453	8,2	5,4	1,6	0,4	0,2
18 То же	А. П. Кульба	1954	262	1,3	1,3	—	—	—
19 То же	Л. С. Матвеев	1955	59	1,9	—	1,9	—	—
20 Сухая ИЭМ им. Гамалеи (из штамма 15-восстанов- ленного)	Л. С. Матвеев	1955	205	22,8	12,6	20,4	2,4	—
21 То же	Н. Г. Олсуфьев О. С. Емелья- нова, В. С. Силь- ченко и др.	1958	6 716	5,9	1,3	3,7	1,0	0,2
22 Сухая ИЭМ им. Гамалеи (из штамма 155)	Они же	1958	6 606	2,8	0,4	2,1	0,5	0,1

1. Type of vaccine; 2. Author; 3. Year of observation; 4. Number of persons inoculated; 5. Percentage of persons with associated reactions; 6. This includes; 7. Enlargement of the lymph nodes; 8. Subjective complaints; 9. Temperature elevation; 10. Loss of the ability to work; 11. Gayskiy virus vaccine; 12. Liquid egg yolk vaccine; 13. The same; 14. Dry NIEG vaccine; 15. Dry Smolensk IEM vaccine; 16. Dry IEM imeni Gamaleya vaccine; 17. Dry IEM imeni Gamaleya vaccine (from the reconstituted 15 strain); 18. Dry IEM imeni Gamaleya

[Legend continued next page]

[Legend continued from previous page] vaccine (from the 155 strain); 19. V. V. Kosmachevskiy; 20. V. S. Sil'chenko; 21. N. A. Kazberyuk; 22. V. S. Sil'chenko; 23. G. P. Uglovoy; 24. V. A. Yudenich; 25. G. P. Uglovoy; 26. A. P. Kul'ba; 27. L. S. Matveyets; 28. N. G. Olsuf'yev, O. S. Yemel'yanova, V. S. Sil'chenko and others; 29. The same.

enitis fail to occur; they become sclerotic and, decreasing somewhat in size, they are afterwards felt in the axilla in the form of a dense nodule, even several years after the vaccination. The fibrotic nodules do not cause any trouble to the inoculees; only occasional persons have mentioned that when the tularin test was performed they felt pain in the axillary region with a certain enlargement of the lymph node. It should be noted that the vaccine being put out now, prepared from the reconstituted 15 strain or 155 strain, gives less-pronounced associated reactions, and no cases of fibrotization of the buboes is observed.

The majority of investigators did not observe cases of suppuration after inoculation lymphadenitis; only M. F. Shmuter (1953) reported that in occasional inoculees with very much enlarged lymph nodes suppuration of them was noted. V. S. Sil'chenko (1953), G. P. Uglovoy (1953) and V. A. Yudenich (1953) noted the more frequent occurrence of lymphadenitis in school-age children after the inoculations than in adults. G. P. Uglovoy (1953) determined the fact that the resolution of such cases of lymphadenitis in children occurs more slowly than in adults. He observed lymphadenitis in 21 percent of school children and in only 12.6 percent of adults 21-26 days after vaccination. There are reports of marked post-inoculation reactions in occasional inoculees (adults).

M. F. Shmuter (1953) described a severe exudative erythema in two inoculees. In them, on the seventh-ninth day after vaccination plaques filled with exudate the size of a 10-kopeck coin appeared on the skin of the arm (site of application of the vaccine). Two-five days after the time of appearance of the "first" erythematous eruptions new areas of skin lesions appeared in the neck, forearm, and arm (the opposite one), and to a lesser degree, on the trunk and lower extremities in the form of individual plaques or areas of confluent erythema the size of the palm or larger. Frequently, a symmetrical arrangement of the erythema was noted, particularly on the extremities. Secondary eruptions were accompanied by a temperature elevation to 38-39°, and at the end of seven-10 days the temperature dropped; the plaques remained for three weeks. The author believes that the oc-

currence of a severe reaction in the inoculees depended on a pronounced allergic state in these persons (the existence of asthma) and believes that inoculations are contraindicated in such persons. R. Ya. Bondar' (1957) observed two cases of severe reactions in the form of an elevation of body temperature, the appearance of multiple areas of skin erythema, malaise, etc. in two persons inoculated percutaneously. These phenomena developed in the third week after the inoculation and should be related to the excessive sensitization of the organism (allergy). A similar case of an allergic reaction (but less severe) was described by E. N. Belostotskaya and coauthors (1955). In such cases antiallergic preparations should be prescribed (dimedrol [benadryl], vitamin C and others).

As has been pointed out in Chapter IX, increased side-effects produced by dry tularemia vaccine (just as in the case of the Gayskiy virus vaccine) in a number of cases where the result of excessive residual virulence of the Gayskiy 15 vaccine strain, particularly during the first years of its use for preparing the vaccine. In 1946-1948 associated reactions were noted, on the average, in 32 percent in persons inoculated percutaneously with the dry NIEG vaccine and in 24 percent of those inoculated with the liquid yolk vaccine. Subsequently, with reduction of the residual virulence of the vaccine strain there is some reduction in the side-effects produced by the tularemia vaccine. After the original properties of this strain had been reconstituted in the tularemia laboratory of the IEM imeni Gamaleya (1955), the side-effects of the vaccine were increased somewhat. Subsequently, serial production of the dry vaccine was developed which cause side-effects in no more than six percent of the inoculated persons (see Table 30).

A. D. Zlatkovskiy and coauthors (1947) relate the degree of expression of the body reactions to inoculation to the dose of vaccine administered during the vaccination. Thus, after the subcutaneous injection of 50,000,000 microbes he observed a temperature elevation in 62 percent of the inoculees; after 12,500,000, in only 1.8 percent. N. A. Gayskiy and coauthors (1949) and P. N. Burgasov (1950) believe that if there is an increase in the degree to which the vaccine is rubbed into the skin the frequency of occurrence of axillary lymphadenitis is increased in the inoculees.

An analysis of the causes of side-reactions in the inoculees permits us to draw the following conclusions: 1) the main cause of pronounced reactive phenomena in a number of inoculees consists of the constitutional features of their bodies (increased reactivity); 2) in some cases dry as well as liquid yolk vaccine have been produced (separate series) with higher side-effect production; 3) improper dilution of the dry vaccine (the use of small doses of the solvent) has led to an

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increased concentration of microbes in the preparation injected at the time of vaccination; 4) a larger number of scratches than is provided for by the instructions or arbitrarily prolongation of them and application of a larger quantity of vaccine (three-four drops instead of two) led, correspondingly, to overdosage with the vaccine; 5) inadequately careful selection of people before inoculation and as the result, the vaccination of persons who had previously been sick with tularemia and those in whom the inoculations were contraindicated. The practice of mass vaccination of people with living tularemia vaccine has shown that with observance of the vaccination technique and the proper selection of people inoculations with this vaccine occur without pronounced associated reactive phenomena, and the population readily offers itself for vaccination against tularemia.

#### Immunological Reactions in Inoculees

As was pointed out in Chapter IX, B. Ya. El'bert and N. A. Gayskiy (1941), M. M. Faybich and T. S. Tamarina (1946), I. N. May-skiy (1953), N. G. Olsuf'yev (1953) and others, on the basis of numerous experiments on laboratory animals supplemented by observations on people, came to the conclusion that a persistent and strong immunity against tularemia is elaborated in the body not only after having had the disease but also after immunization with living tularemia vaccine, and the allergic reaction and agglutinins in the blood represent the indices of immunity. N. A. Gay-skiy (1944) pointed out that the allergic phenomenon in those inoculated with living tularemia vaccine is the main index of immunity as well as the criterion of suitability of the vaccine, correctness of its dosage and the effectiveness of the inoculations. B. Ya. El'bert (1946), based on the fact that the tularin test is an index of a person's having had tularemia in the past and simultaneously of a persistent and prolonged immunity, used this reaction for judging the specific protection of inoculees against tularemia. In the majority of the inoculees, just as in the case of those who have had the disease, he observed a positive allergic reaction. M. M. Faybich and T. S. Tamarina (1946) noted that living tularemia vaccine in the inoculees causes the appearance of the allergic reaction and the agglutination reaction. They found that the time of occurrence of the allergic reaction in those inoculated with dry tularemia vaccine is the same as the time of appearance of this reaction in patients with tularemia. These data made it possible for N. A. Gayskiy, B. Ya. El'bert, M. M. Faybich and later other investigators to make extensive use of immunological reactions (chiefly the tularin test) in those inoculated both for the determination of the effectiveness of the vaccine and for the detection of

the allergic state and the degree of preservation of immunity in the inoculees.

**The Allergic Reaction.** N. A. Gayskiy pointed out that in those inoculated subcutaneously once with living tularemia vaccine the allergic reaction increases, beginning with the first week after vaccination and continuing until the end of a month. Its appearance in the inoculees was observed beginning with the fifth day; by the 30th day it was positive in 100 percent of the inoculees. Afterwards, the allergy remained for quite a long time. N. D. Altareva (1949) noted that from the second week to the end of a month the allergic reaction becomes positive in all inoculees, building up in intensity at this time and subsequently remains at a high level for six-six-and-a-half years (the observation period).

B. Ya. El'bert and coauthors (1946) reported that after percutaneous vaccination allergy develops after five-seven days and is positive in all the inoculees.

M. M. Faybich and T. S. Tamarina (1946) found allergic reactivity of the skin a month after vaccination in all those vaccinated. Approximately the same data were obtained by other investigators (Table 31).

Table 31

Time of Development of Skin Allergic Reactivity after Inoculation

1. Автор	2. % людей с аллергической реактивностью через:				
	3. 7-10 дней	11-15 дней	16-20 дней	21-25 дней	26-30 дней
4. А. Казберук . . . .	—	—	—	—	97,3
5. А. Мирошниченко . . . .	—	100,0	—	—	—
6. С. Сильченко . . . .	69,6	—	—	100,0	—
7. И. Царев . . . .	—	—	—	—	92,0
8. Ф. Шмутер . . . .	70,0	—	92,5	—	—
9. А. Юденич . . . .	—	96,3	100,0	—	—

1. Author; 2. % of people with allergic reactivity after; 3. Days; 4. N. A. Kasberyuk; 5. M. A. Miroshnichenko; 6. V. S. Sil'chenko; 7. M. I. Tsareva; 8. M. F. Shmuter; 9. V. A. Yudenich.

The majority of investigators has come to the following conclusions: 1) in those inoculated with both dry and liquid living tularemia vaccine, beginning with the first week after inoculation, an allergic

reorganization of the body develops which at the end of the month is well expressed in almost all persons vaccinated and is readily determined by means of the tularin test; 2) the presence of an allergic reaction in the inoculees makes it possible to use extensively the tularin test method for determining immunological reorganization of the body after vaccination.

The majority of authors, in the performance of the intradermal test, have used the generally accepted method, injecting 0.1 cc of tularin. In the literature there are recommendations that larger doses be used. P. N. Burgasov (1949) believes that for the purpose of detecting the allergic reaction in those inoculated it is necessary to inject five or even 10 times these doses of tularin. M. L. Khanin (1950) used tularin in a dose of 0.2 cc. M. S. Vasil'yeva and coauthors (1952) injected the inoculees with 0.2-0.3 cc of tularin and thereby obtained pronounced associated reactions.

In generalizing on the considerable material (numbering tens of thousands of inoculees) on the use of tularin according to the generally accepted method we can state that tularin in a dose of 0.1 cc gives clear-cut and very distinct results for judging the presence and degree of preservation of the allergic skin reactivity in the inoculees. Having observed a general body reaction and the presence of necrosis of the skin (temperature elevation to 38° or higher, swelling of the regional lymph nodes, etc.) even after the injection of 0.1 cc of tularin in a number of inoculees, we consider increase in the dose of tularin inadvisable for intradermal injection in order to avoid more pronounced reactions. Recently, the method of the intradermal tularin test has begun to be used extensively for the demonstration of the segment of the population immune to tularemia. Intradermal injection of tularin in mass investigations is difficult because of the relative complexity of this method (work with a syringe) and the general associated reactions encountered thereby, which lead to refusal of the examination (particularly a second one) by a number of people.

According to the data of I. N. Mayskiy (1953), on examination of the inoculees nine months after vaccination, in 10 percent of the persons necrosis of the tissues was noted at the site of intradermal injection of tularin; in three percent, a chill with a temperature elevation to 38°; in 14 percent, malaise and headaches; in 29 percent, a densification and slight enlargement of the regional lymph nodes.

This brings to mind the percutaneous method of the tularin test suggested by A. A. Vol'ferts (1934) and undeservedly forgotten. N. A. Popov and coauthors (1953, 1958) and then N. G. Olsuf'yev and coauthors (1955, 1958) conducted the testing of percutaneous tularin on a larger scale than A. A. Vol'ferts, L. M. Khatenever and others, and

in 1955, on the suggestion of N. G. Olsuf'yev, V. P. Borodin, A. P. Koroleva, Yu. A. Myasnikov, A. M. Prudnikova, O. V. Ravdonikas, V. S. Sil'chenko, A. M. Sorina, L. N. Tormasova and others (1956) investigated more than 8,000 inoculees, who had been sick with tular-emia as well as those who had not been sick and had not been inoculated (with the aim of a control), by means of percutaneous tularin. Thereby, the excellent effect of percutaneous use of tularin was established for the detection of the allergic skin reactivity, and the results of application of percutaneous tests coincided with those of intradermal tests and there was a considerable reduction of side-reactions from the performance of the percutaneous tests. According to the data of these authors, on the examination of 3,867 inoculees (one-eight years after vaccination) a positive skin allergic test was obtained in 91.2 percent; side-reactions were noted in a total of 1.8 percent, whereby necrosis was not found in a single case at the site of application of tularin to the skin. The performance of percutaneous tests with tularin in people who had not been sick with tularemia in the past and who had not been inoculated gave a negative result, which when a positive reaction was obtained in inoculees and in those who had had tularemia confirmed the specificity of the preparation. N. G. Olsuf'yev and coauthors (1955) recommended that tularin be applied to the skin of the middle third of the arm (left arm), because this area is less traumatized and contaminated than the forearm, where some investigators have performed the test according to the principle of the Pirquet test.

The fully successful testing of percutaneous tularin on a large number of inoculees afforded the basis for investigators to recommend it for use instead of the intradermal method in general practice for mass studies of the population for detecting the immune segment, determination of the effectiveness of vaccination, and solution of the problem of the need for giving revaccination. At the present time, percutaneous tularin, produced by the IEM imeni Gamaleya, is used extensively by medical workers.

N. A. Kazbaryuk and coauthors (1956) and I. L. Martinevskiy (1956) recommend that living tularemia vaccine be used instead of tularin for checking the immune state of the organism with the aim of determining the immune segment among the inoculees, applying a single drop of vaccine to the skin of the arm and making two scratches through it. They give as the reasons for their suggestion the degree of expression and frequency of side-reactions observed in inoculees after the performance of the intradermal test, the greater sensitivity of living vaccine as an allergen (N. A. Kazbaryuk) as well as the fact that in performing tests with the vaccine simultaneously immunization of people with reduced immunity and non-immune persons occurs.

This suggestion was not supported, chiefly because of the fact that among the numbers of those being tested there would be persons who had had tularemia in whom an increased allergic reactivity is noted to injection of the living vaccine (V. S. Sil'chenko, 1957; N. G. Olsuf'yev, 1958). On the other hand, in non-immune persons the injection of half the dose of vaccine (one drop) cannot create a full-scale immunity, which requires the revaccination of certain persons in a shorter period than five years. The use of live tularemia vaccine as an allergen can be justified to some degree in the presence of the considerable side-effect production of intradermal tularin, causing pronounced reactions in part of the inoculees and particularly in those who have had the disease. After the testing and incorporation of percutaneous tularin into practice, a preparation which produces practically no side-effects in the inoculated persons and which causes slight reactions in those who have had tularemia, the suggestion of N. A. Kazberyuk and I. L. Martinevskiy that living tularemia vaccine be used as an allergen cannot be accepted.

Above it has been noted that the allergic skin reactivity of inoculees is preserved for a long time, which goes into years. The founders of vaccination against tularemia with living vaccines, B. Ya. El'bert and N. A. Gayskiy, believe that the allergic reorganization in the inoculees is preserved for three-five-and-a-half years after vaccination (the observation period). Further investigations have not only confirmed these data but have also determined the fact that in a considerable number of those inoculated the allergic reactivity is found even later (Table 32).

V. S. Sil'chenko (1956) examined 133 persons 10 years after vaccination, and in 76.8 percent found preservation of the allergic skin reactivity, whereby in a considerable number of the inoculees it was well expressed (from 0.5 to three centimeters).

In the press works have been published in which it has been stated that in the inoculees the skin allergic reactivity is quite rapidly lost. M. S. Vasil'yeva and coauthors (1952) found an allergic reaction in only 71 percent of the inoculees a month after vaccination; in 50 percent, after six months; in only 10 percent, after a year. R. Ya. Chernina (1950) did not obtain a positive allergic reaction 15 months after inoculation in a single person inoculated. R. Ya. Bondar' and coauthors (1952) found an allergic reaction in only 50.2 percent of the inoculated persons examined 10 months after vaccination. In all cases, people were checked who had been inoculated with the liquid yolk vaccine. It must be supposed that the data of R. Ya. Chernina, R. Ya. Bondar' and M. S. Vasil'yeva and others concerning the rapid extinction of the allergic reactivity in the inoculees were the result of application of an



Table 32

## Duration of Preservation of Allergic Skin Reactivity after Inoculation

Автор ①	② % людей с кожной аллергической реактивностью через:						
	1 год ③	2 года ④	3 года ⑤	4 года ⑥	5 лет ⑦	6 лет ⑧	8 лет ⑨
④ М. А. Мирошниченко . . . . .	—	99,5	100,0	100,0	—	—	—
⑤ Ю. А. Мясников . . . . .	—	—	—	100,0	—	—	—
⑥ В. С. Сильченко . . . . .	98,7	98,2	96,3	92,0	90,5	89,1	83,3
⑦ Г. П. Угловой . . . . .	—	—	81,4	75,2	—	—	—
⑧ с соавт. . . . .	92,2	87,4	86,4	—	82,3	75,4	58,3
⑨ Г. П. Угловой . . . . .	—	100,0	100,0	100,0	—	—	—
⑩ М. И. Царева . . . . .	—	—	—	—	—	—	—
⑪ В. А. Юденич . . . . .	90,6	88,0	84,2	73,3	—	—	—

1. Author; 2. % of people with allergic skin reactivity after; 3. Year(s)  
 4. M. A. Miroshnichenko; 5. Yu. A. Myasnikov; 6. V. S. Sil'chenko;  
 7. G. P. Uglovoy and coauthors; 8. G. P. Uglovoy; 9. M. I. Tsareva;  
 10. V. A. Yudenich.

incomplete vaccine which had partially lost its immunogenic properties and in some cases as the result of untimely application of it or incorrect vaccination technique. This was confirmed by the data of R. Ya. Chernina, who used two- and three-months-old vaccine which gave a total of 41 percent successes of vaccination. This is also indicated by observations of G. P. Uglovoy and coauthors (1953) who obtained the most heterogeneous result from the investigation of persons inoculated with liquid vaccine in a period of one-and-a-half to eight months after vaccination. According to his data, after inoculations performed correctly with fresh vaccine by workers of the tularemia-control station, the allergic reaction was noted in 82 percent of the inoculees at the time of the check; when the inoculations were given by local medical workers the number of persons reacting positively to tularin dropped to zero.

During the course of observation of the allergic reactivity in the inoculees it was determined that at the end of two years and more the degree of expression of the allergic skin test (according to the area of hyperemia and infiltration) decreases (Table 33).

From Table 33 it is seen that in the course of time the num-

ber of persons who have a pronounced positive allergic skin test decreases; at the same time, there is an increase in the number of persons inoculated who have a less-pronounced reaction (0.5-one centimeter). However, in a number of inoculees even in the remote periods after inoculation (four-10 years) the reaction remained quite distinct, and the performance of the percutaneous or intradermal test with tularin in some of them was associated with a pronounced general reaction of the body, that is, it occurred in the same way as in those who had previously had tularemia. This speaks for the fact that we cannot differentiate inoculees from those who have had tularemia by the allergic reaction alone.

Table 33

Degree of Expression of Allergic Reaction to the Intradermal Injection of Tularin in Those Inoculated at Different Times after Vaccination  
(after V. S. Sil'chenko)

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① Срок проверки после вакцинации	② % лиц с интенсивностью реакции (участок покраснения и отека в см)				
	0.5	1	2	3	4 и больше ③
20-30 дней ④	0.9	13.1	28.1	32.7	25.2
3-4 года ⑤	4.6	31.7	25.5	23.9	14.3
10 лет	20.8	40.6	30.2	8.4	—

1. Time of check after vaccination; 2. % of persons with the following reaction intensity (area of reddening and edema in centimeters); 3. Or more; 4. Days; 5. Years.

There are data in existence which indicate the lesser persistence of the immunological reorganization of the body in children from seven to 14 years of age inoculated against tularemia. In them an earlier extinction of the allergic skin reactivity is observed than in adults (Table 34) and an earlier extinction of the agglutination reaction (see below).

It should be taken into consideration that in part of the inoculees the allergic reaction to tularin does not appear in the first 24 hours, as occurs shortly after vaccination, but rather after 36-48 hours during the performance of the percutaneous and intradermal tests with tularin in the remote periods after vaccination (two-three years or more). In these cases a doubtful or what is considered to be a negative

Table 34

**Comparative Data on Time of Preservation of Allergic Reactivity in Children and Adults Inoculated against Tularemia**

1 Автор	2 Год опубликования	3 Срок наблюдения (годы)	4 % лиц с кожной аллергической реактивностью	
			5 Дети	6 Взрослые
7 Ю. А. Мясников . .	1953	1	90,9	95,0—100,0
8 Р. Н. Бондарь . . .	1954	2	34,3	83,2
9 Н. А. Казберюк . .	1953	2	67,0	76,1
10 Г. П. Угловой . . .	1953	6	65,0	75,0
11 В. С. Сильченко . .	1956	8	73,6	86,4

1. Author; 2. Year of publication; 3. Observation period (years); 4. % of persons with allergic skin reactivity; 5. Children; 6. Adults; 7. Yu. A. Myasnikov; 8. R. Ya. Bondar'; 9. N. A. Kazberyuk; 10. G. P. Uglovoy; 11. V. S. Sil'chenko.

test becomes quite distinct at 48 hours. This should be taken into consideration in the performance of percutaneous and intradermal tests with tularin; the check of the results (reading of the reaction) should be made twice: after 24 and after 48 hours. Without observance of this rule an error may be made in the evaluation of the presence of immunity in the inoculees.

With respect to the problem of comparative evaluation of the effectiveness of percutaneous and subcutaneous methods of application of the vaccine in people we can present the data of M. L. Khanin (1950), who used the dry NIEG vaccine. According to his material, among persons inoculated percutaneously 93.8 percent reacted positively to tularin at the end of the year (114 persons were checked), while among those inoculated subcutaneously 78.1 percent reacted at the time of a check made after six months (32 persons were checked). Based on these data, Khanin considers the percutaneous method of vaccination more effective. Similar results were obtained by A. A. Selezneva (1949). In the group of persons vaccinated percutaneously 85.6 percent reacted to tularin after six months; in the group of those vaccinated subcutaneously, 75 percent. The groups included 200 and 400 persons each who had been inoculated with the NIEG vaccine. The

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agglutination test was positive after six months in 30 percent of the first group; the serum titers amounted to 1:5-1:40; in the second group 19 percent; the serum titers were 1:10-1:20. A year after the inoculations in the group of those vaccinated subcutaneously only 69.9 percent reacted to tularin; in the group of those inoculated percutaneously, the data on the number of those reacting to tularin after a year were not reported.

**Agglutination Test.** As has been determined by B. Ya. El'bert and N. A. Gayskiy (1941), M. M. Faybich and T. S. Tamarina (1946) and others, in those inoculated with living tularemia vaccine an allergic reorganization of the body occurs and agglutinins are found in the blood, but the latter appear somewhat later than the occurrence of allergic reactivity. B. Ya. El'bert and coauthors (1947) found agglutinins in the inoculees beginning with the 17th day after vaccination. According to his data, on the 50th day the agglutination test was positive in 99.3 percent of the inoculees, whereby the serum titers varied from 1:20 to 1:1280. N. A. Gayskiy (1947) found agglutinins in the blood of inoculees beginning with the 20th day after vaccination, and by the 30th day they were found in all inoculees; in part of them (seven percent) the agglutination titer was high (1:1000). M. M. Faybich and T. S. Tamarina (1946) a month after vaccination also observed the agglutination reaction in all the inoculees. N. G. Olsuf'yev (1953) according to materials of a number of tularemia-control stations, pointed out that one-three months after vaccination agglutinins are found in the blood in all those who have been inoculated with a positive result, usually in a titer of 1:50-1:100; less often, 1:400 and higher.

According to the data of V. S. Sil'chenko, V. A. Yudenich and others, agglutinins in a titer of 1:50-1:100 begin to be found in the blood of inoculees on the ninth-10th day after vaccination, and by the 21st-25th day they are found in 90-92 percent (Table 35). These data are in full agreement with the results obtained by N. A. Gayskiy, B. Ya. El'bert and M. M. Faybich. An exception is constituted only by the observations of A. A. Selezneva (1948), who found agglutinins in the inoculees in exceptionally early periods after vaccination, as early as on the second-third day. The serum titer, true enough, was low at this time, 1:5-1:80. It may be supposed that this was the result of subcutaneous injection of the dry NIIEG vaccine which possessed quite a high degree of activity. In the literature no such early periods of detection of agglutinins in the serum of inoculees have been published by other authors.

The development of the positive agglutination test in the inoculees occurs in the same way as in patients with tularemia, lagging only with respect to the degree of its expression. B. Ya. El'bert and

Table 35

Time of Appearance of Agglutination Reaction after Inoculation

1 Автор	2 % людей с агглютинами через:						
	3 1-5 дней	3 6-10 дней	3 11-15 дней	3 16-20 дней	3 21-30 дней	3 31-40 дней	3 41-60 дней
4 Н. А. Казберюк	-	-	-	-	84,0	-	-
5 А. А. Селезнева	+	100,0	-	-	-	-	-
6 В. С. Сильченко	-	+	4,8	42,4	90,3	-	98,3
7 М. Ф. Шмутер	-	-	-	-	+	-	78,6
8 В. А. Юденич	-	+	-	-	92,4	-	-

Note. The plus sign indicates the time (beginning) of the examination and the obtaining of a positive reaction. 1. Author; 2. % of people with agglutinins after: 3. Days; 4. N. A. Kazberyuk; 5. A. A. Selezneva; 6. V. S. Sil'chenko; 7. M. F. Shmuter; 8. V. A. Yudenich.

coauthors (1947), investigating inoculees and patients with tularemia, noted that in the inoculees by and large a moderate agglutination reaction is observed -- in 70 percent the serum titer does not exceed 1:160; in the persons who have had tularemia, on the other hand, about 70 percent have reactions with high agglutination titers (1:640 and higher), which has found confirmation in the observations of subsequent authors (V. S. Sil'chenko, 1953, and others). In those inoculated, as well as in patients with tularemia, the blood agglutinins appear in the first week after vaccination, but they are found in a titer of 1:50-1:100 or higher only in the second-third week. At the end of the fifth-seventh week the agglutination titer reaches a maximum and then begins to fall. However, final appearance of the agglutinins in the inoculees does not occur quickly, and the reaction, true enough in low titers -- 1:10-1:100 (less often, 1:200 or higher) -- is found several years after the vaccination (Table 36).

Therefore, agglutinins in the blood of the inoculees are found six years after vaccination, and according to recent data (V. S. Sil'chenko, 1957) even after eight-and-a-half years. This should be taken into consideration by physicians making the diagnosis of tularemia. Considering the large number of persons inoculated against tular-

Table 36

## Length of Time Agglutinins are Found in the Blood after Inoculation

Авторы ①	② % лиц с агглютинами через:					
	1 год ③	2 года	3 года	4 года ④	5 лет	6 лет ⑤
④ Н. А. Казберюк . . . . .	72,6	64,7	—	—	—	—
⑤ И. Л. Мартиневский . . . . .	85,7	85,4	—	69,6	—	—
⑥ П. Угловой с соавт. . . . .	70,0	—	—	—	—	—
⑦ В. С. Сильченко . . . . .	84,6	68,1	61,9	56,8	52,0	48,6
⑧ В. В. Слесаренко и С. Г. Бу- яло . . . . .	—	—	—	—	45,0	—
⑨ М. Л. Ханни . . . . .	88,6	76,1	—	—	—	—
⑩ М. Ф. Шмутер . . . . .	98,2	76,1	—	—	—	—
⑪ В. А. Юденич . . . . .	83,4	77,0	61,2	52,6	—	—

1. Authors; 2. % of persons with agglutinins after: 3. Year(s); 4. N. A. Kazberyuk; 5. I. L. Martinevskiy; 6. G. P. Uglovoy and co-authors; 7. V. S. Sil'chenko; 8. V. V. Slesarenko and S. G. Buyalo; 9. M. L. Khanin; 10. M. F. Shmuter; 11. V. A. Yudenich.

emia (in the USSR tens of millions have been inoculated) and the duration of preservation of agglutinins in the blood of some of them, a physician very often can encounter a positive agglutination test in a patient being examined (with the tularemia diagnosticum). Therefore, a single positive agglutination test alone in a low titer in the absence of epidemiological or clinical data cannot serve as a criterion for making the diagnosis of tularemia. For the correctness of differentiation of tularemia from other diseases a second performance of the test (it should be performed two or more times) is needed for checking on the increase in the agglutinin titer (see also Chapter VIII).

The characteristics of the agglutination test in children inoculated against tularemia have been studied by only occasional authors. They have noted that this reaction, just like the skin allergic reactivity, is extinguished earlier in children than in adults. N. A. Kazberyuk (1953), two years after vaccination, found agglutinins in the blood of adults in 64.7 percent of the cases; in children, in only 51.6 percent of the cases checked. Whereas in the adults the agglutination titers of

the sera reached 1:80, in children they did not exceed 1:40. As has been indicated above, the skin allergic reactivity in the inoculees is preserved for a longer time than agglutinins can be found in the blood (Table 37).

Table 37

Length of Time the Agglutination and Allergic Reactions are Maintained after Inoculation

1 Сроки проверки	2 % лиц, у которых обнаружены			
	3 аллергическая реакция по данным		4 реакция агглютинации по данным	
	5 В. С. Сильченко	6 В. А. Юденича	5 В. С. Сильченко	6 В. А. Юденича
7 до 6 месяцев	100,0	100,0	95,1	—
8 1 года	99,5	90,6	84,6	83,4
9 2 лет	97,9	88,0	68,1	77,0
10 3 "	95,9	84,2	61,9	61,4
11 4 "	93,2	—	56,8	—
12 5 "	88,8	—	51,0	—
13 6 "	85,3	—	48,6	—

1. Time of checking; 2. % of persons in whom the following were found: 3. Allergic reaction according to the data of: 4. Agglutination reaction according to the data of: 5. V. S. Sil'chenko; 6. V. A. Yudenich; 7. Up to; 8. Months; 8. Year; 10. Years.

Six years after vaccination the allergic reaction in the inoculees is encountered two times more often than the agglutination reaction. These data are evidence of the fact that agglutinins in the blood of inoculees stop being found somewhat earlier than the skin allergic reactivity is extinguished. However, as has been indicated previously, in a number of persons inoculated agglutinins can be found in the blood even at the end of six-eight years. Therefore, by means of the agglutination test alone or the allergic test alone it is impossible to differentiate inoculees from those who have had tularemia in the past, although in the latter the agglutination titer of the serum is higher (during the first few months after the disease) as a rule. For this, it is necessary to analyse the data of the history and epidemiology and to have a good registration of those inoculated against tularemia. In the course of time the agglutination titer of the sera in the inoculees (just

as in those who have had the disease) decreases and the relatively high quantities of antibodies found in the first months after vaccination are no longer observed (Table 38).

Table 38

Changes in the Agglutination Titer of Sera in Inoculees in the Remote Periods after Vaccination

Авторы	Год опуб- лико- вания	Средние титры сыворотки через					
		1 месяц	1 год	2 года	3 года	4 года	5 лет
6. Я. Бондарь с со- авт. . . . .	1952	1:80— 1:100	1:20— 1:100	—	—	—	—
7. И. С. Тинкер и Т. И. Пучкова . . . . .	1948	1:320	—	1:20— 1:40	—	—	—
8. В. В. Слесаренко и С. Г. Буяло . . . . .	1956	1:92	1:129	—	—	—	1:10— 1:80
9. В. А. Юденич . . . . .	1953	1:65	1:36	1:25	1:11	—	—
10. Г. П. Угловый . . . . .	1953	—	1:40	—	—	1:10— 1:20	—
11. Л. Мартиневский . . . . .	1955	—	1:76	1:59	—	1:35	—
12. А. Казберюк . . . . .	1953	1:40	1:32	1:23	—	—	—
13. М. Ф. Шмутер . . . . .	1953	1:68	1:41	1:13	—	—	—
14. В. С. Сил'ченко . . . . .	1953	1:184	1:114	1:96	1:78	1:67	1:43
15. М. А. Мирошниченко . . . . .	1953	1:221	1:74	—	—	—	—
16. Л. С. Матвейетс с со- авт. . . . .	1957	1:59	—	—	—	—	—

1. Authors; 2. Year of publication; 3. Average serum titers after:  
4. Month; 5. Year(s); 6. R. Ya. Bondar' and coauthors; 7. I. S. Tin-  
ker and T. I. Puchkova; 8. V. V. Slesarenko and S. G. Buyalo; 9.  
V. A. Yudenich; 10. G. P. Uglovoy; 11. I. L. Martinevskiy; 12. N.  
A. Kazberyuk; 13. M. F. Shmuter; 14. V. S. Sil'chenko; 15. M. A.  
Miroshnichenko; 16. L. S. Matveyets and coauthors.

Therefore, agglutination titers in the inoculees decrease from year to year, and at the end of the observation period (five years) agglutinins are found in the majority of inoculees in serum dilutions of 1:10-1:50. Therefore, the agglutination test can be used as an auxiliary method for determining the duration of the immunological reorga-  
nization of the bodies of inoculees.



**The Opsonocytophagic Reaction.** Based on the statements made by L. M. Khatenever (1943), A. F. Bilibin (1943) and others concerning the essential importance of phagocytosis in the infectious process (in tularemia) and the increase of the intensity of the phagocytic reaction during the course of vaccine therapy of patients with tularemia, some authors have used the opsonocytophagic test for determining the time of occurrence of immunity and the preservation of it in those inoculated with tularemia vaccines. N. A. Gayskiy and coauthors (1947) established the fact that the phagocytic reaction in the inoculees appears early, almost simultaneously with the allergic reaction, beginning with the third day after vaccination. During the first two weeks it is slightly positive; after 30 days it is marked in all inoculees. Later, N. A. Gayskiy and coauthors (1949) noted that after 30 days the opsonocytophagic reaction is positive in 92 percent of the inoculees.

Afterwards, the opsonocytophagic reaction in the inoculees was studied by N. D. Altareva (1949), M. L. Khanin (1950), V. A. Yudenich (1954) and others. It was noted that this reaction is found in the inoculees up to six-and-a-half years after vaccination (the observation period), whereby after three years it is positive in more than 80 percent of the persons investigated.

We should like to note that the technique of performing the opsonocytophagic reaction is more complicated than that of the intradermal test and particularly more complicated than that of the percutaneous test with tularin, as the result of which this test, which is of definite scientific research interest, cannot be used on a broad scale.

**The Complement-Fixation Test.** The complement-fixation test in inoculees was studied by I. N. Mayskiy (1949) and V. A. Yudenich (1953). They established its earlier appearance than the agglutination reaction and they found that it appears in higher titers (three times or more). In noting the considerable advantages of the complement-fixation test, its higher degree of sensitivity, making it possible to detect minimum quantities of antibodies in the sera of inoculees, the authors point out the complexity of performance of the test which interferes with utilization of it in general practice.

#### **Hematologic Changes after Vaccination**

Blood changes occurring in people as the result of vaccination have been studied by only occasional authors. By and large, they consist of moderate leukocytosis, monocytosis, and acceleration of the sedimentation rate. I. L. Martinevskiy (1953), examining inoculees on the 10th, 17th and 24th days after vaccination, noted a certain increase in lymphocytes, monocytes and eosinophils in the differen-

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tial count. The lymphocytosis increased until the 24th day, and in various inoculees (2.6 percent) reached 32-35 percent. He observed the appearance of Turk's cells in the blood. N. A. Kazbaryuk (1953) points to a relative increase in the lymphocyte count with a certain reduction in the neutrophil count. The erythrocyte sedimentation reaction was accelerated. L. I. Rod'kina (1954) observed a slight leukopenia, increase of the lymphocytes (to 40-60 percent) with simultaneous reduction in the segmented neutrophils (on the average, to 48 percent); the monocyte account was slightly increased or remained unchanged. The sedimentation rate in part of the inoculees was accelerated. V. A. Yudenich (1953) noted a slight leukocytosis and increase in the number of monocytes to 15-18 percent from the fifth through the 30th day after vaccination in the case of positive inoculations.

Therefore, changes in the blood of the inoculees (lymphocytosis, increased sedimentation rate and others) somewhat resemble the changes observed in patients with tularemia but are less pronounced.

#### Epidemiological Effectiveness of Vaccination

The Antiepidemic Effectiveness of Vaccination. N. A. Gayskiy, in 1942-1943, used living virus vaccine in foci of tularemia in Kazakhstan, Kirovskaya and Voronezhskaya oblasts. The inoculations were given at a time when outbreaks of tularemia had already subsided and only occasional fresh cases among people were recorded. During this period, in various inhabited places the majority of inhabitants from five to 56 years of age was inoculated. The fact that the inoculations were actually given after the outbreak had stopped did not permit N. A. Gayskiy to make observations of the prophylactic effectiveness of the vaccination, but it was still noted that after the vaccination had been conducted there were no cases among the inoculees. In one inhabited place where the vaccination was given, cases of tularemia again appeared after eight-nine months, but there were no cases among the inoculees.

A more distinct effect of vaccination in foci of tularemia is found in the report of B. Ya. El'bert, who along with I. S. Tinker, T. I. Pushkova and others, in 1945, organized inoculations against tularemia in 24 inhabited places (B. Ya. El'bert and coauthors, 1947). Inoculations and observations of their effectiveness were conducted at the time of a developing agricultural outbreak of tularemia. People who were most exposed to the danger of infection were included in the vaccination (agricultural workers). After the conclusion of vaccination there were only isolated cases of tularemia among the inoculees in the first two weeks (that is, at a time when an adequately strong immunity

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had not been created as yet in the inoculees). In all, 0.63 percent of the inoculees became sick and 4.3 percent among the persons who were not inoculated. In two kolkhozes almost complete vaccination was accomplished which aborted an outbreak. This was the first experience in the extensive study of the antiepidemic effect of living tularemia vaccine, which confirmed the high value of vaccination of people during an epidemic outbreak of tularemia. A very clear-cut antiepidemic effect of the inoculations was then established in outbreaks of arthropod-borne tularemia (V. P. Borodin, 1950, 1958), water-borne (A. A. Selezneva, 1948), agricultural and domestic (V. A. Yudenich, 1957) as well as other types. V. S. Sil'chenko (1952) clarified the fact that mass inoculations against tularemia conducted directly before the occurrence of an outbreak completely protect people against the disease, and inoculations conducted at the beginning or at the time of a developing outbreak completely stop the cases of disease among people in the next 12-15 days. We should like to present striking examples of the antiepidemic effectiveness of vaccination according to our observations in Voronezhskaya Oblast in 1946-1953.

1. In the winter, in one of the kolkhozes of Paninskiy Rayon, on the third-fifth day of threshing cereal crops which were in stacks in the field, group "influenza-like" cases occurred among the participants. On detailed investigation of the patients tularemia was found. In the stacks a large number of sick rodents (common voles) and their bodies were found. The threshing was temporarily stopped. After complete vaccination of the population of the kolkhoz was carried out threshing of the grain from the same stacks was started again. Despite the fact that the epizootic among rodents had assumed an even greater scale and the number of dead bodies increased there were no more cases among the participants of the threshing who had been inoculated (Fig 72).

2. In Povorinskiy Rayon on the territory of one inhabited place a tularemia epizootic occurred among mouse-like rodents which were in the stacks, ricks and on the barnyard floors where the threshing had been carried out. Cultures of the tularemia pathogen were isolated from the rodents which died. Whereas among the uninoculated the majority of members of the brigade became sick with tularemia, of 22 inoculated persons (vaccination was carried out three months before beginning threshing) no one became sick.

3. In one of the villages of Mikhaylovskiy Rayon toward autumn a large number of mouse-like rodents appeared. In October a tularemia epizootic was found among them. Shortly after, cases of tularemia began to be recorded among people who had been in contact with agricultural products. During a period of five days the entire

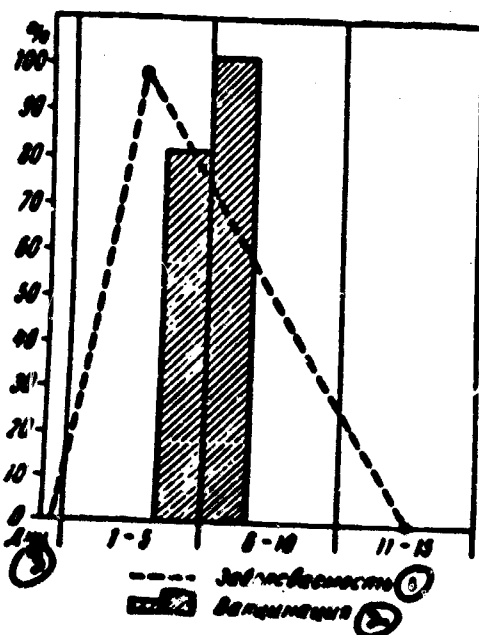


Fig 72. Effectiveness of Vaccination During Outbreak of the Agricultural (Threshing) type. 1. Morbidity rate; 2. Vaccination; 3. Days.

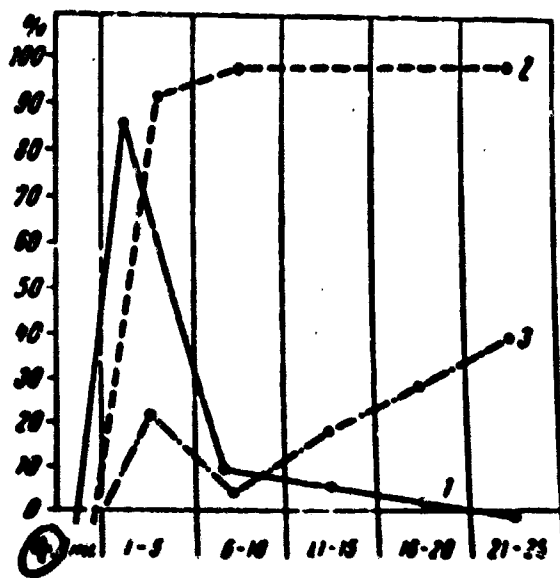


Fig 73. Effectiveness of Vaccination in an Outbreak of the Domestic Type. Tularemia morbidity rate (1) and vaccination (2) compared with the morbidity rate in another place where no vaccination was carried out (3). 4. Days.

population of the village was inoculated with liquid yolk vaccine. During the first two weeks after the completion of vaccination only isolated cases of tularemia were found among the inoculees; after this time, not a single case of tularemia was detected. In the nearby inhabited places, where vaccination was not carried out in time tularemia was observed among people for a number of months afterwards (Fig 73).

4. In two adjacent rayons -- Borinskiy and Vasil'yevskiy -- the same epidemiological situation was created: a large number of common voles and house mice appeared not only in the fields but also in inhabited places. In the winter, a tularemia epizootic occurred among the rodents. Cases of tularemia in people also occurred; infection occurred through contact with the rodents and their bodies as well as food products and water infected by the rodents. In these regions

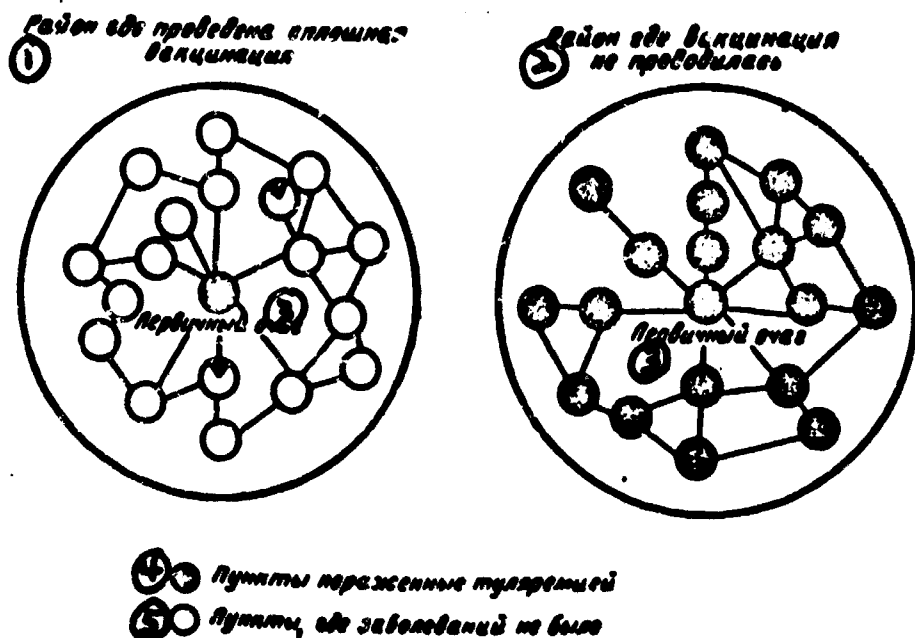


Fig 74. The Effect of Continuous (Ring Method) Vaccination on Reduction of the Incidence of Tularemia. 1. Rayon in which continuous vaccination was conducted; 2. Rayon in which vaccination was not conducted; 3. Primary focus; 4. Places infected with tularemia; 5. Places where there were no cases.

conditions were created for mass spread of tularemia. In Borinskiy Rayon vaccination was conducted by the ring method in a short period, whereby almost the entire population was inoculated not only in the area of the primary focus of tularemia but also far beyond its limits in other inhabited places. In the adjacent Vasil'yevskiy Rayon the inoculations were not organized in time. Whereas in the first rayon the outbreak of tularemia was rapidly and completely eliminated, in the second one it continued to develop and spread to neighboring inhabited places (Fig 74).

Different results were obtained when vaccination was conducted in an untimely way, slowly, or where only small separate population groups were inoculated. Although the cases of disease stopped among the inoculees in these cases also such vaccination did not give a true antiepidemic effect (because of the small immune segment) and the outbreak dragged on for a number of months.

In one of the villages of Mikhaylovskiy Rayon an outbreak of tularemia developed, first of an agricultural (threshing) and then of a domestic type of mouse origin. Vaccination was begun only in the second month after the onset of diseases among people, was conducted slowly, so that at the end of the third month only 64 percent of the population had been inoculated. By this time a decline in the epidemic outbreak had already been noted in the inhabited place, and the vaccination gave no perceptible antiepidemic effect (Fig 75).

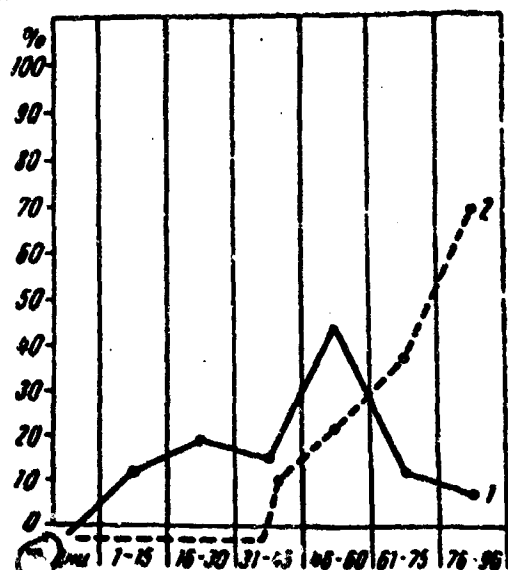


Fig 75. Movement of Tularemia Morbidity (1) after Slow Vaccination (2); 3. Days.

For greater clarity we are presenting the comparative data of the morbidity rate dynamics from tularemia in Bobrovskiy and Kashirskiy rayons. In the former mass vaccination was conducted against tularemia; in the latter, it was not (Table 39).

In Bobrovskiy Rayon, even in the third five-day period, the incidence of disease as the result of the inoculations given decreased by three times; in the fourth and fifth five-day periods, by more than 30 times, and at this time it was almost completely eliminated. In Kashirskiy Rayon, where inoculations were not given, the morbidity rate continued to be high at the end of a month.

Materials of mass observations accumulated at the present time (B. Ya. El'bert, N. G. Olsuf'yev, I. N. Mayskiy, V. P. Borodin, N. A. Kazberyuk, Yu. A. Myasnikov, O. V. Ravdonikas, V. S. Sil'chenko, G. P. Uglovoy, M. I. Tsareva, M. F. Shmuter, V. A. Yudenich and others) have established the high degree of antiepidemic value

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Table 39

The Influence of Inoculations against Tularemia in Reducing the Morbidity Rate in People During an Epidemic Outbreak of Tularemia

Срок наблюдения (дни) ①	Бобровский район (проведена массо- вая вакцинация) ②		Каширский район (вакцинации не проводилась) ③	
	число случаев ④	%	число случаев ④	%
⑤ 1-5-й (до начала прививок)	142	100,0 <sup>1</sup>	94	100,0
⑥ 6-10-й (в Бобровском рай- оне привито 89,7% насе- ления)	136	96,3	110	117,2
⑦ 11-15-й	48	34,2	116	123,1
16-20-й	4	2,9	99	106,0
21-25-й	2 <sup>2</sup>	1,2	79	84,6
26-30-й	1	0,9	77	82,7

⑧ Заболеваемость в первую пятидневку для обоих районов принята за 100 %.  
⑨ Заболеваемость в Бобровском районе после 20-го дня учета зарегистрирована только среди непривитых.

1. Observation period (days); 2. Bobrovskiy Rayon (mass vaccination conducted); 3. Kashirskiy Rayon (no vaccination conducted); 4. No. of cases; 5. First-fifth (before beginning the inoculations); 6. Sixth-10th (in Bobrovskiy Rayon 89.7 % of the population was inoculated); 7. 11th-15th; 8. The morbidity rate in the first five days is taken as 100% in both rayons; 9. The disease in Bobrovskiy Rayon was recorded only among the non-inoculated up to the 20th day.

of application of living tularemia vaccine in foci of tularemia. Complete vaccination of people in an area of a tularemia focus gives a notable reduction of the morbidity rate as early as the first eight-10 days, and after 12-15 days it leads to complete elimination of the outbreak. Vaccination of 90-95 percent of the population in the region of an epidemic focus actually aborts the outbreak, because after this only isolated cases of disease are found among the non-inoculated. A particularly good effect in the control and prophylaxis of tularemia is given by the ring method of vaccination proposed by V. S. Sil'chenko (1952), in which mass inoculations are conducted simultaneously in the place

affected by tularemia as well as in the inhabited places located around the epidemic focus. Mass vaccination of people provides a good prophylactic effect when there is a danger of tularemia of any origin: agricultural, occupational, arthropod-borne, food product, water, etc. For the purpose of complete and rapid achievement of the antiepidemic effect vaccination should be conducted on a large scale (with coverage of 100 percent of the population which needs to be inoculated) and in a short time (three-five days).

#### The Prophylactic Effectiveness of Planned Vaccination.

Several years ago inoculations against tularemia were conducted as an emergency antiepidemic measure organized directly at the time of occurrence of tularemia among people or else when a tularemia epizootic was found among rodents. Such vaccination gave good results, but the effect frequently was incomplete, because before the beginning of vaccination part of the people had already become sick with tularemia. Also cases were observed among those who had been inoculated in which an adequately strong immunity had not yet managed to be formed to withstand the infection. At times, it was difficult to cover the entire groups of people who had to be covered by the inoculations in a short period, particularly agricultural workers during the period of summer and autumn field operations. There was also a delay in the bringing of the vaccine into areas of epidemic outbreaks. Untimely giving of the inoculations reduced the antiepidemic effect of vaccination.

After positive results of experimental studies had been obtained and after the fact that the immunological reactions were maintained for a long time (years) in inoculated persons and the high degree of antiepidemic effectiveness of vaccination during outbreaks of the disease had been clarified, various investigators set about the study of the prophylactic effectiveness of vaccination, using as a basis the absence of tularemia among inoculated workers in tularemia laboratories and among those inoculated persons who lived in the area of natural tularemia foci.

N. A. Gayskiy (1946) made an observation on 11 workers in the tularemia department of the Irkutsk Plague-Control Institute who had been vaccinated with the tularemia virus vaccine. Despite the fact that these persons worked under situations of close contact with virulent cultures of *B. tularensis* from four to 28 months, none of them became sick with tularemia. Before the introduction of vaccination against tularemia into practice the majority of workers in the laboratories usually became sick with tularemia. N. A. Gayskiy made another observation (1946) in a tularemia focus, where a year before this vaccination against tularemia had been carried out. In this case also, among the inoculees, there were no cases of tularemia, whereas among



the non-inoculated there were cases of disease. The observation of N. A. Gayskiy (1944) on a group of workers at the Irkutsk Plague-Control Institute inoculated against tularemia and subjected to experimental infection with a virulent culture of the tularemia microbe six months after vaccination was particularly significant proof. Inoculation immunity in them was quite strong, and none of them became sick with tularemia. I. S. Tinker and T. I. Puchkova (1948) made an observation for one-and-a-half years on six workers in the tularemia laboratory who had been vaccinated against tularemia. They also noted that despite the close contact of these workers with infectious material none became sick with tularemia (see also Chapter XI).

All these observations were made in a relatively short time (from four to 28 months) and on a small number of inoculees, but the good results (absence of cases among the inoculees) permitted putting the question of conducting prophylactic vaccination against tularemia as a planned matter outside of an epidemic or epizootic period.

In 1947 V. S. Sil'chenko suggested and accomplished the giving of mass planned prophylactic inoculations against tularemia along with workers in the Voronezhskaya Oblast Tularemia-Control Station, Ye. N. Yezhova and V. G. Klenova. The inoculations were given according to the following principle: 1) in the area of natural foci of tularemia the entire population was vaccinated solidly, regardless of the kind of occupation, beginning with the age of four in order to create a maximum immune segment prior to the occurrence of a tularemia epizootic among the rodents; 2) outside of the area of natural foci those persons were inoculated who by virtue of their working conditions could be in contact with infected material and rodents -- the sources of the tularemia infection (those who caught water rats and other rodents, rat killers, hunters, workers in pelt storehouses, etc.); 3) workers in tularemia-control institutions who worked constantly in a region of natural foci of tularemia as well as in the laboratory with virulent cultures of the tularemia microbe and were subjected to the everyday threat of infection were inoculated.

Then, the tularemia-control station proceeded with complete planned prophylactic vaccination of the population not only in various places but also in entire rayons, including 96-99 percent of the population in the inoculations. The observation was made for eight years and included a considerable number of inoculees. During this period there was not a single case of tularemia among them, including among laboratory workers who had worked for all these years with material known to be infected. At the same time, among persons who had not been inoculated and who had been under conditions of less contact with infectious material cases of tularemia were noted. Subse-

sequently, such inoculations were organized and carried out in a number of krays and oblasts in the periods between epizootics and epidemics. The inoculations were of a mass nature (tens and hundreds of thousands of persons were vaccinated for prophylactic purposes). This made it possible to study the prophylactic properties of living tularemia vaccine and the duration of the immunity created by it in the inoculees on a broad scale.

In the study of the results of planned prophylactic vaccination over a period of four-six years N. G. Olsuf'yev, I. N. Mayskiy, G. P. Uglovoy, M. I. Tsareva, Yu. A. Myasnikov and others concluded that it was highly effective. According to the conclusion of N. G. Olsuf'yev (1953), prophylactic vaccination made it possible to eliminate almost completely the disease tularemia not only within the limits of various inhabited places and rayons but in entire krays and oblasts, and at the present time planned vaccination in the USSR is already a generally accepted method of tularemia prophylaxis. We should like to present examples of the effectiveness of prophylactic vaccination.

In Voronezhskaya Oblast for a number of years outbreaks of tularemia of the occupational, arthropod-borne and water-borne origina were observed every year. The population which lived in the area of natural foci of this infectious disease was almost completely vaccinated (98.6 percent). A comparison of the morbidity rate in the area of these foci for six years before vaccination and for the same period after vaccination showed that the morbidity rate decreased by almost 60 times after the inoculations (Fig 76). The disease observed here after the inoculations occurred exclusively in those who had not been inoculated. Tularemia outbreaks observed prior to that time in the Oblast had been aborted as the result of the vaccination. Subsequently, only isolated cases of the disease were noted among those who had not been inoculated.

V. A. Yudenich (1953) reported that in Smolenskaya Oblast there were natural foci of tularemia in the area of which virulent cultures of the tularemia pathogen had been isolated repeatedly from rodents and ticks. After conducting vaccination for four years (the observation period) cases of tularemia were no longer observed among those inoculated, but among those who were not inoculated they still occurred.

In a number of rayons of Voronezhskaya Oblast water rats had been caught for trade purposes since 1936. In the past the water rat trappers, as a rule, became sick with tularemia during the first or one of the subsequent hunting seasons. The members of their families, who assisted in processing and drying the pelts, also became sick. In the past seven years after carrying out complete vaccination

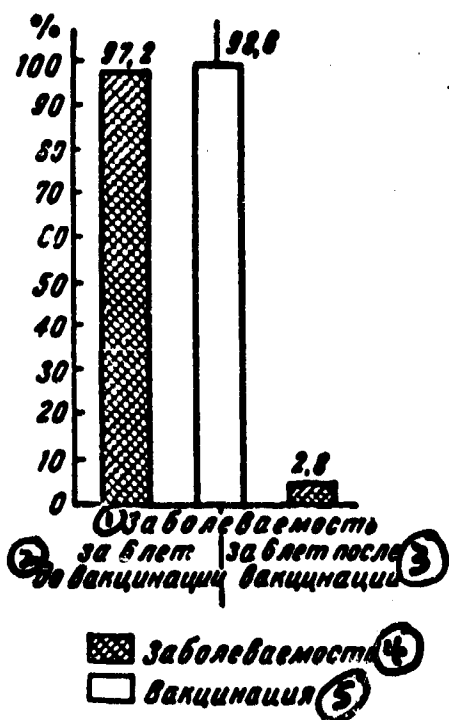


Fig 76. Effectiveness of Vaccination in a Region of Natural Tularemia Foci in Voronezhskaya Oblast. 1. Morbidity rate; 2. In the 6 years before vaccination; 3. In the 6 years after vaccination; 4. Morbidity rate; 5. Vaccination.

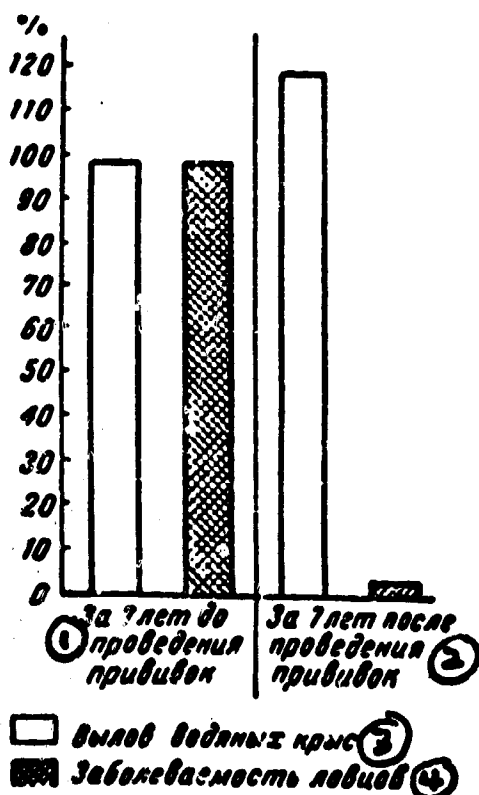


Fig 77. Incidence of tularemia in Water Rat Catchers before and after Inoculations Given in Voronezhskaya Oblast. 1. For 7 years prior to giving inoculations; 2. For 7 years after giving inoculations; 3. Catching water rats; 4. Morbidity rate in the trappers.

of the trappers and their family members the incidence of tularemia among them was zero, despite the fact that during this period the trapping of water rats had increased somewhat. During these years tularemia epizootics were observed among the water rats and virulent cultures of *B. tularensis* were isolated. A significant proof as a test was the disease found in two water rat trappers who had come from an adjacent oblast and who had not been given vaccination against tularemia

(Fig 77).

Natural foci of tularemia in Povorinskiy Rayon of Voronezhskaya Oblast had been demonstrated about 30 years ago; their effects are continuing to be manifested at the present time. This has made it necessary to conduct complete vaccination in this Rayon of the rural and city population. Railroad workers and white-collar workers were, in part, not vaccinated. Cases of tularemia in people occurred for eight-and-a-half years after conducting the vaccination in the Rayon; solely those who had not been inoculated, chiefly railroad groups, became sick. The investigation revealed the presence of a large immune segment among the population (up to 80 percent) because of the vaccination conducted in 1946-1948.

G. P. Uglovoy, I. N. Mayskiy, N. G. Olsuf'yev and others (1953) established the fact that those who had been inoculated and who had been in contact with infected material five years after vaccination did not become sick with tularemia, whereas those who were not inoculated did become sick. According to the data of V. S. Sil'chenko, in two adjacent rayons -- Berezovskiy and Novo-Usmanskiy -- mass vaccination of the population was conducted. Five years after this, in the region of natural foci, in the soddy-alluvial meadows hay-harvesting was conducted, whereby along with a large number of local kolkhoz members (who had been inoculated) brigades worked here made up of city workers (who had not been inoculated). In both rayons cases of tularemia in people occurred which were of the ulcerative-bubonic form (arthropod-borne infection), exclusively among those who had not been inoculated in brigades coming from the city.

The study of the prophylactic effectiveness of planned vaccination against tularemia has made it possible to draw the following conclusions: 1) prophylactic vaccination conducted in the region of natural tularemia foci with living tularemia vaccine leads to a complete stoppage of the disease among those who have been inoculated for a period of six-eight years (the observation period); 2) inoculations reliably protect even those workers who work with virulent cultures of the tularemia microbe and with material known to be infected for seven-eight years; 3) after conducting the mass vaccination of people (95-97 percent) outbreaks of tularemia are no longer being observed in the area in which the inoculations were conducted; only sporadic cases are encountered among those who have not been inoculated; 4) the results of prophylactic vaccination (absence of tularemia among the persons inoculated for five-eight years) confirm the duration and strength of the immunity conferred on the inoculees as the result of vaccination; 5) carrying out planned prophylactic vaccination against tularemia has justified itself completely; therefore, public health organs should as

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a planned matter organize and conduct complete vaccination of people who are in the region of natural tularemia foci or who by the nature of their activity are subjected to the danger of infection.

#### Incidence of Tularemia in the Inoculated

The main criterion of the effectiveness of vaccine prophylaxis, particularly the duration and strength of the inoculation immunity, is the absence of disease among the inoculees. We can consider those vaccines reliable and highly effective which create good protection against infection for a long time in the inoculees. In evaluating the effectiveness of tularemia vaccination when it is carried out directly before an outbreak or at the time of disease which has already developed the opinions of Soviet investigators are similar. N. A. Gayskiy, B. Ya. El'bert, M. M. Faybich, N. G. Olsuf'yev, I. N. Mayskiy, I. S. Tinker, A. A. Selezneva and others believe that the inoculations given with living tularemia vaccines quite quickly (in a period of 10-15 days) create a strong immunity in the inoculees, which leads to a cessation of disease among those vaccinated. However, during the first two weeks after vaccination, that is, when immunity is not sufficiently strong in the inoculees and part of the people may have been vaccinated after being infected, in the incubation period, some inoculated persons become sick with tularemia.

B. Ya. El'bert and coauthors (1947) observed tularemia in those inoculated in the first 12 days after vaccination. Later, according to their data, none of those inoculated became sick. V. P. Borodin (1950) also noted disease in the inoculees in a period from two to 10 days; only three persons became sick a month after vaccination. A. A. Selezneva (1948) observed disease in the inoculees until the sixth-seventh day after vaccination. V. S. Sil'chenko (1948) established the fact that inoculees, as a rule, did not become sick later than the 16th day after vaccination. In the majority of cases (77.3 percent) the disease occurred in them during the first week after vaccination. N. A. Kazbaryuk (1953) no longer found disease among the inoculees 10 days after vaccination.

B. Ya. El'bert and coauthors (1947) pointed out that tularemia in the inoculees who became sick in the first few days after vaccination has a much milder course than in persons who have not been inoculated. These observations have been confirmed by the data of V. S. Sil'chenko (1948), V. P. Borodin (1958) and other authors. In the presence of fatal outcomes in patients with tularemia (who have not been vaccinated) within limits of 0.5 percent, not a single death has been recorded among those who have been inoculated and who have

become sick with tularemia in a 10-year period.

Investigators are particularly interested in the problem of the possibility of infection of inoculees with tularemia in the remote periods after vaccination. In the Soviet literature this problem had been discussed very scantily; in the foreign literature we could not find any comparable data of interest to us, because there are no tularemia living vaccines there (similar to ours), and abroad cases among the inoculees are a usual phenomenon (according to the data of Foshay up to 30 percent of those inoculated with killed vaccines become sick).

P. N. Burgasov (1949) observed cases of tularemia among people inoculated with living tularemia vaccine but he does not present specific data and he does not mention the time after vaccination at which the cases occurred. N. G. Olsuf'yev (1953), in accordance with materials of peripheral institutions, reports four cases of disease among inoculated persons approximately a year after vaccination, whereby tularemia was clinically overt in them and was confirmed by the data of a laboratory examination. The infection occurred by the arthropod-borne route. He also pointed to a case of infection of an inoculee six years after vaccination. This case, occurring as the result of a laboratory accident and transfer of a virulent culture to the eye, was then described by O. P. Khizhinskaya and V. M. Stupnitskaya (1956). M. F. Shmuter (1953) points to isolated cases of tularemia in the inoculees occurring five-seven months after vaccination. However, with respect to these people he reports that in the majority of them the vaccination had not been successful (and, therefore, they could not be considered vaccinated); in another group following vaccination a slight inoculation reaction was observed. Ye. I. Kositsyna (1958) reported seven cases of tularemia among the inoculees (with a positive result); the inoculations had been given a year or more before the time of infection.

At the same time, many authors (Yu. A. Myasnikov, M. I. Tsareva, V. A. Yudenich and others) indicate that they have not observed cases of tularemia among inoculees in the remote periods after vaccination (four-five-year observation periods). V. S. Sil'chenko (1953) could find only isolated cases in which the diagnosis of tularemia was confirmed in inoculees in a period of eight years of observation, but in almost all cases it was difficult to learn the quality of the vaccination. In the majority of cases the diagnosis of tularemia proved to be erroneous. It was the result of the fact that the physicians resorted to laboratory examinations at the improper time, limiting themselves to a single agglutination test without considering epidemiological data preceding the disease and, chiefly, forgot about the prolonged preservation of immunological reactions after vaccination. The existence of a positive serological test was made the basis of a diagnosis of tular-

emia, even in the absence of epidemiological data and a clinical picture which was not characteristic. The cause of tularemia in the inoculees in some cases can be infection of man with massive doses of a virulent microbe. Most often, in the inoculees, the disease occurs as the result of improper vaccination, which provides for the production of an inadequate and short-term immunity. This is evidenced by materials of R. Ya. Chernina, R. Ya. Bondar', M. F. Shnuter and others, which indicate the possibility of a quite rapid extinction of immunity in some groups of inoculees (after five-10-15 months).

Therefore, in noting the various cases of tularemia in inoculees as the result of the premature loss of immunity in them or a breakthrough in the immunity by a massive dose of infection, it must be considered that these cases are rare and require further study. In the majority of inoculees (after vaccination with a complete vaccine) immunity is maintained no less than five-six years. With a proper and serious attitude of practising medical workers toward problems of vaccine prophylaxis of tularemia cases among the inoculees can be stopped completely.

In the analysis of the morbidity in inoculees it is essential to analyze every such case with great caution, because there may be people in this group who show allergic reactions to the incorporation of tularemia pathogen into their bodies. N. G. Olsuf'yev and coauthors (1959) noted the occurrence of an allergic reaction in people immunized against tularemia and who had consumed water from brooks naturally infected with the tularemia microbe for drinking purposes. V. P. Borodin and coauthors (1958) described an allergic reaction in two inoculees infected with *Rhipicephalus rossicus* ticks (a virulent culture of *B. tularensis* was isolated from the ticks). In these persons a brief temperature elevation (up to two days) and an enlargement of the regional lymph node were noted. Such cases, if a careful examination is not made, can readily be considered tularemia occurring in a latent or abortive form.

#### Revaccination

Since inoculations began to be used extensively against tularemia in the practice of public health organs the problem of the time of conducting revaccination and the indications for it has maintained special current importance. This is conditioned by the presence of the danger of infection of the population living in the area of natural tularemia foci. The founders of application of living tularemia vaccine, N. A. Gaydaryuk and B. Ya. El'bert, pointed out that this vaccine creates a stable and strong immunity for a long time in the inoculees.

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N. A. Gayskiy (1947) believed that the duration of immunity after tularemia vaccination is similar to the time immunity lasts after vaccination against smallpox. B. Ya. El'bert (1946) calculated the period of immunity in those inoculated with liquid yolk vaccine to be no less than three-four years. P. N. Burgasov (1950), on the basis of the observations by M. M. Faybich and A. D. Zlatkovskiy, wrote that the duration of immunity (as determined by the preservation of the allergic reorganization of the body in the inoculees) is more than three-and-a-half years.

Initially, when the time a sufficiently strong immunity to tularemia had not been studied accurately and the immunizing properties of various series of vaccines, particularly yolk, underwent variations under the influence of various causes, many authors recommended that revaccination be conducted every one or two years. At the present time, observations have been accumulated which have been conducted over a much longer period (six-eight years) and on a large number of people. Thereby, it was possible to establish a direct relationship between the preservation of immunological reactions in the inoculees (allergic reactivity) and the absence of disease among them and, therefore, the duration of a strong immunity. It was made clear that a stable and strong immunity in the majority of inoculees is preserved no less than five-six years (according to recent data an even greater duration of the inoculation immunity is noted). M. I. Tsareva and N. K. Mal'tseva (verbal communication) point out that in all the inoculees whom they checked (51 persons) six years after vaccination the allergic skin reactivity was preserved, and none of the inoculees became sick with tularemia. G. P. Uglovoy obtained positive results from the tularin test at the end of an eight-year period in a considerable number of inoculees. V. S. Sil'chenko (1957) established the existence of an allergic reorganization of the body and the absence of disease among persons in a group inoculated nine and 10 years before (see also Table 32).

In the light of these observations the problem of the time of revaccination against tularemia should be solved. We cannot give attention to various studies based on occasional groups of inoculees vaccination in whom was conducted without adequate control of the quality of the inoculations, frequently with incomplete vaccine, and as a result of which an extremely rapid subsidence of the immunity was noted, which is not characteristic of those inoculated with complete vaccines. Observations on these latter groups, according to the data of the majority of authors, confirm a five-six-year period of immunity; therefore, a period of five years was adopted by the conference on vaccine prophylaxis of tularemia in 1953 and is provided for in special



instructions [for revaccination] worked out in 1959.

A number of authors have raised the question of the indications for revaccination, whereby the majority of them (N. A. Kazber-yuk, I. N. Mayskiy, V. A. Yudenich and others) believed that revaccination should be carried out only according to the epidemic indications. Without denying the need for considering these indications in the rayons where for a long time there have been no cases among people or epizootics among rodents, other investigators (V. S. Sil'chenko, M. F. Shmuter and others) consider it advisable to conduct revaccination as a planned matter in a region of natural tularemia foci. V. S. Sil'chenko bases this recommendation on the same reasons as those for giving planned inoculations. Some groups of people (water rat hunters and muskrat hunters, receivers of pelts for furs, rat killers and others) are continuously under the danger of infection and tularemia. In the region of natural foci, particularly in places of arthropod-borne outbreaks of tularemia, the epidemiological situation dictates the need for taking appropriate prophylactic measures every year. It would be late if revaccination is begun when epizootics have been found or when cases have appeared among people. Therefore, it is best to conduct revaccination beforehand as a planned matter, but in every individual case following preliminary coordination of this problem with the oblast or kray sanitary-epidemiological station.

At the present time, public health organs have adopted the obligatory planned accomplishment of revaccination every five years. Because vaccination is not always given in a completely adequate manner, it is recommended that the rayon sanitary-epidemiological stations regularly and selectively check the presence of the immune segment among the population, resorting to the percutaneous tularin test for this purpose and investigating 100-200 persons in every inhabited place where there is a danger of infection of people. Such a check should be carried out with deterioration of the epidemiological prognosis and at the same time putting the question of conducting revaccination prior to the time provided for in the instructions. Reduction of the immune segment among those investigated (for the region of tularemia natural foci, to less than 80-75 percent) will serve as a criterion for conducting the revaccination.

The problem of the degree of expression of the allergic reaction in inoculees and the strength of immunity has not been conclusively solved. There are suggestions in existence to the effect that in various inoculees at the end of a five-year period even in the presence of a positive tularin test the immunity can become so much weakened that it stops protecting the inoculees against cases of tularemia. This problem requires further detailed study.

The technique of revaccination is not at all different from the technique of vaccination both with regard to preparation (disinfection) of the skin and the number of scratches and drops of vaccine to be applied (four scratches, in two rows and two drops of vaccine for every person being revaccinated). In the case of revaccination of people who have a strong immunity (after vaccination or after having had a case of tularemia) a quite distinct inflammatory reaction appears at the site of percutaneous injection of the vaccine, but in contrast to that in non-immune persons it develops as early as 24 or 48 hours later, that is, it is accelerated, and is basically of an allergic nature (V. S. Sil'chenko, 1952; I. N. Mayskiy, 1953; G. P. Uglovoy, 1953; M. F. Shmuter, 1953; N. G. Olsuf'yev, 1953, 1958; V. A. Yudenich, 1953, and others). The reaction consists of the fact that a reddening and edema having a transverse diameter of 0.5-one centimeter and sometimes more appear at the site of scarification of the skin. In part of the persons revaccinated, on the second-fifth day, a papule or vesicle (less often a pustule) forms along the courses of the scratches with the subsequent formation of small scabs by the eight-ninth day (M. F. Shmuter, 1953, and others). Revaccination conducted in persons who have maintained their vaccine immunity has been called "superimmunization" by N. G. Olsuf'yev, distinguishing it from revaccination (that is, reimmunization) of persons in whom the vaccine immunity has been lost. It should be noted that in the case liquid yolk vaccine is used for revaccination (superimmunization) in a number of cases (up to one-third) the reaction to the inoculation does not develop at all, which was not observed after the use of the dry NIEG vaccine (M. F. Shmuter, 1953).

According to the data of a mass check made in 1958 by the tularemia laboratory of the IEM imeni Gamaleya in conjunction with institutions at the periphery and including more than 5,000 persons, it was established that a reaction of the allergic type after revaccination (superimmunization) with vaccines made of the Gayskiy reconstituted 15 strain or the Yemel'yanova 155 strain occurs in practically 100 percent of the cases and is maintained until the sixth day in almost all (99.9 percent) of those revaccinated. Therefore, for the purpose of facilitating the work of medical workers it is recommended that the results of revaccination be checked at the same time as the results of initial vaccination are checked (that is, for the first time between the fifth and seventh day after inoculations). Previously, it was recommended that such a check be made on the second-fifth day after revaccination. The presence of an allergic reaction in those revaccinated is considered a positive result, which is evidence of the preservation of immunity in the inoculee. According to the data of the same check,

loss of the reaction in a small part of those revaccinated (superimmunized) was observed beginning with the eighth day; by the 12th-15th day the reaction had disappeared in approximately 20 percent; by the 20th-22nd day, in the majority.

In the opinions of various investigators, in the case of percutaneous revaccination of immune persons a vaccine process may be superimposed on the allergic reaction because of the partial survival and multiplication of the vaccine (M. F. Shmuter, 1953; G. P. Uglovoy, 1953, and others), which justifies the use of the term "superimmunization". A check showed that in such persons an intensification of immunological reactions is noted, particularly an increase in the agglutination titer of the serum. Attention was directed to this for the first time by M. F. Shmuter (1953) and subsequently his data were confirmed in the investigations of a number of authors (V. S. Sil'chenko, 1953; M. V. Vasil'yeva and coauthors, 1953; I. L. Martinevskiy, 1955; L. S. Matveyets, 1960, and others). An increase in the serum titer is usually noted in persons revaccinated a year or more after vaccination, but may be observed in the earlier periods also.

The data of G. P. Uglovoy are particularly interesting; he determined the fact that in persons who have been revaccinated (superimmunized) the immunity is maintained for a longer time than in those who have been vaccinated. In accordance with his data, of 42 persons subjected to vaccination followed by revaccination a year later, 36 persons, that is, 85.5 percent, reacted to percutaneous tularin after seven years. For the purpose of comparison 76 persons vaccinated simultaneously with the previous group, living in the same locality, but not given revaccination were used. In the check made simultaneously with the first group only 49 persons, which amounts to 65.2 percent, reacted to percutaneous tularin, that is, 20 percent less than in the group which had been revaccinated. Vaccination and revaccination were conducted with the percutaneous NIEG vaccine; only adults were included in the investigation (G. P. Uglovoy, personal communication).

In the revaccination of persons who have lost their immunity completely at the time of revaccination the vaccine process in them develops at the same time and occurs with the same intensity as in persons being immunized for the first time (V. A. Yudenich, 1954; L. S. Matveyets, 1960). Judging by the immunological reactions, the immunity in these persons returns to the level reached at the time of the first vaccination. V. A. Yudenich and then L. S. Matveyets noted various cases in which at the time of check before revaccination the skin allergic test to tularin was absent (however, after vaccination it had been positive); nevertheless, after revaccination the local inoculation reaction appeared after 48 hours and had a shorter course than at

the time of the first immunization.

Some authors note that at the time of revaccination general associated reactions which are more numerous and more pronounced are observed than at the time of the first vaccination. I. N. Mayskiy (1953) and G. P. Uglovoy (1953) noted a high percentage of people with side-reactions causing in part of them a temporary loss of the ability to work at the time of conducting a revaccination a year after the inoculation. Thus, after five days 50 percent had a densification and enlargement of the regional lymph nodes; 20 percent, malaise and headaches; eight percent, a temperature elevation to  $38^{\circ}$ ; 8.1 percent, loss of the ability to work. V. A. Yudenich (1954) believes that revaccination is associated with an increase in the general reactions particularly in the lymphatic apparatus. He noted that enlargement of the lymph nodes in revaccinated persons is observed six times more often than in the inoculees (pain in the axilla in those vaccinated for the first time was observed in 3.1 percent of the cases; in revaccinated persons, 18.3 percent; correspondingly, lymphadenitis was found in 2.2 percent in the first group and 12.7 percent, in the second). According to his data, there is an increase in the number of persons with temporary loss of the ability to work after revaccination (from zero to 1.4 percent). These data, and particularly the figures obtained by I. N. Mayskiy and G. P. Uglovoy, should set us on the alert with regard to conducting revaccination, but it should be noted that none of the other investigators obtained such high figures of associated reactions at the time of revaccination. N. G. Olsuf'yev (1953), according to the materials of a number of tularemia-control stations, pointed out that revaccination of those who have been inoculated with a well-expressed immunological state causes a moderate local, and in various cases, general reaction, which usually is no greater than that at the time of vaccination. M. F. Shmuter (1953) believes that revaccination is tolerated well even by persons with a marked allergic reactivity. He observed practically no general reactions at the time of revaccination. In conducting experimental revaccination the more frequent occurrence of associated reactive phenomena (30.9 percent) was noted than at the time of vaccination (23.2 percent), but in the majority of persons these phenomena were poorly expressed and did not interfere with the working capacity of the revaccinated persons (Table 40).

The fact attracts attention that revaccination conducted in the early periods after the initial inoculation produces side-reactions in a considerably larger number of persons than more remote revaccination (Table 41).

In conducting the revaccination medical workers will always encounter people who to varying degrees have been sensitized to

Table 40

## Associated Reactions in Those Inoculated and Revaccinated

① Характер реакции	② % лиц с реакциями	
	③ при вак- цинации	④ при ре- вакцина- ции
⑤ Субъективные жалобы (сла- бость, потемнение, го- ловная боль и т. п.) . . .	13,9	17,2
⑥ Повышение температуры до 37,6° . . . . .	4,8	6,6
⑦ Подмышечные лимфадене- ты . . . . .	4,5	7,1

Note. Revaccination was carried out 13-72 months after inoculation. 1. Nature of the reaction; 2. % of persons with reactions; 3. At the time of vaccination; 4. At the time of revaccination; 5. Sub-  
jective complaints (weakness, malaise, headache, etc.); 6. Tempera-  
ture elevation to 37.6°; 7. Axillary lymphadenitis.

the tularemia microbe (tularemia antigen); therefore, just as in the case of performing the test with tularin, in a number of persons there will be a reaction of the body of the allergic type.

In carrying out the revaccination at the time provided for in the instructions (that is, after five years), however, the reactivity of the organism is already considerably reduced and there will be no reason for expecting pronounced side-reactions. Such reactions may be encountered only in occasional persons being revaccinated.

An extensive check of the degree of side-effect production of the vaccines now being prepared for revaccination (superimmunization) which was organized in conjunction with the peripheral institutions of the tularemia laboratory of the IEM imeni Gamaleya showed better tolerance of these vaccines than of those previously put out. Where vaccines made of the Gayskiy 15 reconstituted strain were used associated reactions were noted in 20 percent of those being revaccinated; where vaccines made of the 155 strain were used, they were found in only nine percent, being, on the average, 15 percent.

The Voronezh Tularemia-Control Station has carried out the mass revaccination of people living in an area of natural tularemia foci as a planned matter. Revaccination was conducted with dry tularemia

Table 41

The Incidence of Associated Reactions as a Function of the Time of Conducting the Revaccination

Характер реакции (1)	(2) % лиц с реакциями через		
	(3) 1 год	3 года	(4) 5-8 лет
(5) Субъективные жалобы	20,0	6,3	15,2
(6) Повышение температуры	8,0	4,8	5,3
(7) Регионарные лимфадениты	50,0	12,7	5,1
(8) Трудопотери	8,1	1,4	0,3-0,5

#### Notes.

1. Data are given by I. N. Mayskiy and G. P. Uglovoy for a year; by V. A. Yudenich, for three years; by V. S. Sil'chenko, five-eight years. 2. It must be supposed that persons who had previously suffered from tularemia fell into the group of those revaccinated by I. N. Mayskiy and G. P. Uglovoy, which can be the explanation for such high figures of side-reactions. 1, 2. [Same as Table 40]; 3. One year; 4. Five-eight years; 5. Subjective complaints; 6. Temperature elevation; 7. Regional lymphadenitis; 8. Work loss.

vaccine in a period between five and eight years after vaccination. There were more than 35,000 persons revaccinated. An analysis which we made of the materials of this planned revaccination confirmed the data of the experimental revaccinations, namely: a) early development of skin reactions of the allergic type in those revaccinated; b) good tolerance of the vaccine at the time of revaccination by the majority of those revaccinated and the absence of pronounced associated (side) reactions in them; c) a good prophylactic effect from the revaccination conducted. At the time of conducting the revaccination it was possible to detect those who had previously not been inoculated and to increase the immune segment of the population to high levels.

#### Organization of Inoculations

The organization and giving of inoculations against tularemia

should be based on a study of the regional characteristics of the epidemiology and epizootology of tularemia in the area in which the inoculations are conducted. The problem of organizers of vaccine prophylaxis lies in conducting and completing it before a period in which infection can occur in the presence of unfavorable epizootological prognosis. For this purpose it is necessary to study beforehand the epidemiological situation in the locality being accommodated, to demonstrate the existence of natural foci of disease and their localizations, to establish the seasons in which the disease occurs and the groups mainly subjected to the danger of infection (from the nature of their activity, from their being close to the region of natural foci, etc.). It should also be determined (approximately) how many people need vaccination. Only after carrying out such preliminary work can a plan of effective vaccine prophylaxis be made up properly. In places where there are natural foci of tularemia it is necessary to make up a prospective plan of complete vaccination of the entire population in addition to the plan of operation of the vaccination (in the presence of a large number of people who need to be vaccinated and where it is impossible to conduct this work in a single season or year).

The experience of work of antiepidemic organizations has shown the expediency of centralization of the entire matter of vaccine prophylaxis. Peripheral therapeutic-prophylactic and sanitary-antiepidemic institutions, on the basis of a study of the epidemiological situation in their own regions, as well as on the basis of a consideration of groups of people who need to be vaccinated, have presented the oblast (or kray) sanitary-epidemiological station with requests that inoculations be conducted with designation of the number of persons noted and the time (the month or quarter) in which the vaccination is to be given. This station, as represented by the department of particularly dangerous infections, takes into consideration and summarizes all the requests made, makes appropriate corrections and additions to them, and on the basis of the epidemiological situation in the oblast (or in the kray) makes up the prospective plan of vaccine prophylaxis for tularemia for the next few years with the aim of achieving maximum coverage of the population with inoculations (in the area of rayons enzootic for tularemia complete vaccination of the population seven and over is being planned). The plan is made up in accordance with the rayon situation. On the basis of this plan the annual plan of inoculations for the current year is made out. The most convenient times for conducting planned mass inoculations against tularemia are the first and fourth quarters.

The plan made up in accordance with every rayon is sent to the corresponding rayons by the oblast sanitary-epidemiological

station beforehand (a copy of the general plan for the given rayon is sent out) to the head of the public health department and the chief physician of the rayon sanitary-epidemiological station for discussion and accomplishment of the necessary preparation work. The directors of the rayon public health organizations (the rayon health department, sanitary-epidemiological station, rayon hospital) make up their own vaccination plans, noting in them definite places and groups which need vaccination as well as the time at which the vaccinations are to be given. Simultaneously, medical workers are appointed responsible for the given areas in the matter of inoculations. A group of inhabited places is assigned to every medical worker and there he will carry out the vaccination. Medical workers, prior to beginning vaccination, assemble at the rayon center for the purpose of receiving detailed instructions on vaccination technique. Such instructions should be given with a practical illustration of vaccination technique in people who are to be inoculated or else with medical workers who have not yet been inoculated. Workers of the antiepidemic organization are subsequently obliged to make a check of the inoculations and record their results in the various localities.

If there is a problem of conducting the vaccination as an emergency antiepidemic measure (the presence of an epizootic among rodents, detection of cases of tularemia among people, etc.), a brigade is organized (there may be several of them) from medium-level medical workers, headed by one of the physicians of the medical institution or sanitary-epidemiological station. In organizing such a brigade it should be taken into consideration that a single medical worker can easily inoculate about 100 persons in making rounds of an inhabited place per workday. Therefore, a brigade of 10 persons will inoculate 3,000 persons in three days, and if the vaccinations are to be concentrated in some single place (school, institution, medical aid station), even more. In any case, in the organization of inoculations for emergency epidemiological indications vaccination should be conducted in as short a time as possible (three-five days).

A good antiepidemic effect is given by the use of the ring method of vaccination. In view of the fact that the disease can spread to a number of adjacent localities from the initial focus of the infection in a short period of time vaccination should be planned and carried out not only in the place affected at the given moment but also in other settlements located 10-15 kilometers around it (sometimes even more), even though not a single case of tularemia has been noted in them. In this method of vaccination, conducted rapidly and extensively, tularemia is localized to the initially-affected place, although the epizootic among rodents is noted even beyond its limits.



At the present time, planned prophylactic inoculations against tularemia have become quite widespread and it should be noted that they have played an important part in a marked reduction of tularemia in the USSR. Instructions for giving prophylactic inoculations against tularemia (1959) provide for extensive accomplishment of prophylactic inoculations as a planned matter, whereby continuous vaccination of the population is provided for in rayons and inhabited places previously unfavorable with respect to tularemia as well as in places where there are active natural foci of tularemia. Simultaneously, it is recommended that vaccination of some groups of people who by virtue of their activity are exposed to the danger of infection with tularemia, for example, workers in enterprises which process agricultural products, rodent pelts, etc., and then of persons who come for a short time into rural localities enzootic for tularemia, etc. (for more details see Chapter XI) as a planned matter.

In rayons which have previously been favorable for tularemia (within limits of oblasts and krays which have had cases of tularemia in the past) the inoculations are given according to the epidemic indications. The indications for the necessity of vaccination are an increase in the rodent census -- the main sources of infection (the common vole, house mouse, water rat, steppe lemming, etc.) -- which assumes the nature of mass multiplication, the detection of a tularemia epizootic in rodents, cases among people, and a delay of threshing of cereal crops before the winter comes. In the case of a particularly unfavorable epidemiological situation (danger of mass infection of people, particularly in the case of outbreaks of the water-borne, agricultural and domestic types) children two years and over should be inoculated.

Before vaccination and revaccination it is necessary to detect the people who have previously had tularemia (because they show increased reactions to inoculation with living vaccine) as well as people in whom the inoculations are contraindicated because of their health. The detection of them is conducted on the basis of registration data on those who have had tularemia and patients who are on record in the medical district; this should be carried out by medical workers. With respect to those who have had tularemia, in doubtful cases, recourse should be had to performing the percutaneous test with tularin. Contraindications (by virtue of the state of health) to giving vaccinations with living tularemia vaccine are the same as contraindications to the use of vaccines against other infectious diseases but in view of the slight side-effect production of tularemia vaccine they may be limited, particularly during the period of a real danger of occurrence of a tularemia outbreak, because mortality from tularemia is usually observed in elderly persons with cardiac and pulmonary diseases.

In view of the fact that in some regions inoculations have to be given against tularemia, brucellosis, anthrax, smallpox, intestinal and other infectious diseases, the problem has arisen of the possibility and expediency of simultaneous vaccination of people against a number of infectious diseases. Experiments on laboratory animals have shown that with simultaneous immunization with tularemia and brucellosis as well as plague living vaccine, and with killed cholera and other vaccines, immunity to these components develops normally, without any mutual interference (see Chapter IX). However, in people the possibility of simultaneous vaccination against tularemia and brucellosis has not been studied as yet (R. S. Amanzhulov, 1956, and others). Ye. A. Gubina and G. P. Uglovoy (1958), inoculating 185 persons with percutaneous associated brucellosis-tularemia vaccine, determined its harmlessness and the fact that it "took" successfully (by the skin inoculation test). These authors made it clear that immunological reorganization of the body after the use of associated vaccine occurs in approximately the same way as after separate inoculations against tularemia and brucellosis. These data permit us to consider it possible to use associated vaccination against tularemia and brucellosis with further study of the duration and strength of immunity to both infectious diseases. Inoculations against other infectious diseases, from now until the problem of associated vaccination against them is solved, should be conducted separately from tularemia at intervals of no less than 20 days. After a tularemia inoculation the other inoculations should be given no earlier than a month later.

#### Conclusion

Soviet scientists B. Ya. El'bert, N. A. Gayskiy and M. M. Faybich have illustriously solved the problem of specific vaccine prophylaxis of tularemia. Living tularemia vaccines which they have proposed have been tested by time not only experimentally but also in general practice. The conference on tularemia (1953) convoked by the Ministry of Health RSFSR noted that in the attainment of a major reduction in the incidence of tularemia in foci of this infectious disease the main role has been played by mass prophylactic vaccination of people. We should not be limited to the successes achieved; it is necessary to do further work on the study of the effectiveness of vaccine prophylaxis of tularemia, establishing the maximum period of maintenance of vaccine immunity, particularly after revaccination, and on further improvement of living tularemia vaccine, using it in association with other vaccines, etc.

## Chapter XI

### System of Measures in Foci of Tularemia

#### General Comments

Immunization of people with living tularemia vaccine is a most important prophylactic measure in foci of tularemia because of the exceptional effectiveness of the latter. Various persons have expressed the idea that in the presence of highly effective inoculations against tularemia the prophylaxis of this infectious disease, like that of smallpox, has become a purely organizational problem, and careful use of vaccination alone can completely eliminate tularemia in people in the area of its natural foci. This opinion should be considered erroneous. This problem cannot be solved by vaccination alone if only because part of the population remains non-inoculated as the result of contraindications (by virtue of the state of health) to the inoculations or refusals of the inoculations by various persons. In addition, a certain part of those inoculated can lose their immunity before the time of re-vaccination, that is, before five years (see Chapter X).

In contrast to smallpox, tularemia is an infectious disease with a natural focus and the arrest of cases of the disease in people as the result of vaccination does not signify the health of the natural foci themselves; the potential danger of them continues to exist (V. M. Zhdanov). As practice has shown, mass vaccination of the population very reliably prevents the occurrence of epidemic outbreaks of tularemia or small group cases. However, sporadic cases of tularemia among people from time to time continue to be noted, the more often the broader the contact of the population with rodents or objects contaminated by them in the presence of a diffuse tularemia epizootic among the rodents. Therefore, vaccination of people should be, of necessity, conducted in combination with other measures directed at the elimination of sources of infection and elimination of transmission factors. Tularemia among people can be eliminated completely only as the result of planned use of a System of Measures in Foci of this Infectious Disease. Thereby, the vaccination should be regarded as a temporary and necessary measure, carried out until natural foci of tularemia are localized and then eliminated as the result of conducting broader health improvement measures.

The variety of epizootological and epidemiological manifestations of tularemia makes the prevention and control of it compli-

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cated. In no other disease do we have such a multitude of sources of the infectious disease and routes of transmission of it. Nevertheless, the prospect of eliminating natural foci of tularemia should be considered real under conditions of further growth in the welfare of the Soviet Union on its road toward communism, the material possibilities wherein will be exceptionally great.

### Planning of Measures

The main condition for the success of taking measures against tularemia is comprehensiveness of them, provided for by bringing all interested organizations and departments into the accomplishment of them as well as the use of the workers themselves, because through the forces of medical workers alone the prophylaxis of tularemia cannot be effected properly. In a decree (January 1960) of the CC CPSU and Council of Ministers USSR "Measures for the Further Improvement of Medical Care and Safeguarding of the Health of the Population of the USSR" it was stated that community, agricultural, veterinary, public education organs as well as enterprises, sovkhozes, kolkhozes, administrative and public organizations and the population should participate along with public health organs in taking measures for the reduction and elimination of infectious diseases including tularemia. In accordance with existing directives of the government of the USSR each republic, kray or oblast has to work out the republic (or kray, or oblast) comprehensive plans of measures against tularemia every year (with subsequent approval by the local authorities). On the basis of them rayon (or city) plans are worked out in every rayon enzootic for tularemia as applied to the local conditions.

Comprehensive plans should include measures directed at:

- 1) elimination of sources and vectors of the infectious disease -- rodents (deratization), ticks and other arthropods; 2) reduction in the size of population groups susceptible to tularemia (vaccine prophylaxis); 3) elimination of transmission factors of the infectious disease (general sanitary measures depending on the characteristics of various tularemia outbreaks). Measures for avoiding the importation of tularemia infection to new territories and measures directed at health improvement of natural tularemia foci should be included in the comprehensive plan also. Each of these major divisions in turn can be subdivided into smaller ones: 1) organizational measures (solutions provided by the local Councils of Workers' Deputies, orders for the various departments, checking on their execution, holding of conferences, etc.); 2) investigation measures (recording the census of rodents, ticks, dipterous vectors, investigation of them for tularemia infection, investigation

of the sanitary status of various objects [buildings and structures], checking of the immune segment among various population groups); 3) measures for the direct accomplishment of one division or another (agrotechnical measures, deratization proper, hunting and trapping certain species of rodents, the destruction of ixodid ticks, vaccination, fulfillment of the sanitary norms and elimination of sanitary defects in various objects); 4) measures for the preparation of cadres (preparation of physicians and medium-level medical workers on problems of the clinical aspects, diagnosis and prophylaxis of tularemia, the training of kolkhoz and sovkhos agronomists on rodent control and veterinary workers on the matter of controlling ticks; training of kolkhoz rat eliminators, instruction of sanitation authorities); 5) sanitation education work among the population, popularizing general sanitation problems of tularemia control, problems of vaccine prophylaxis, deratization, tick control, etc.; this division is of very great importance in tularemia considering the high degree of susceptibility of the population to it and the possibility of extensive spread of tularemia epizootics over the territory.

As has been mentioned above, public health organs alone cannot carry out the entire combination of measures against tularemia, in connection with which accomplishment of them is entrusted to a number of ministries, departments and administrations. Thus, rodent control should be conducted by kolkhozes and sovkhoses under the direction of the corresponding organs of the Ministry of Agriculture as well as by institutions of the Ministry of Communications, of the maritime and river fleets, mercantile institutions, committees of government reserves, etc. along with the disinfection service of the public health organs (extermination of rodents in inhabited places). General sanitation measures (including protection against penetration of rodents into buildings) are taken by the same organizations and departments with the participation of the Ministry of Municipal Services and other institutions. The hunting of water rats and other rodents, which are of significance as sources of tularemia infection, is organized by the Tsentrosoyuz [Central Union of Consumers' Cooperatives] through the peripheral organizations subordinate to it. Vaccination of the population is conducted chiefly by public health organs as well as by other departments having medical services. Tick control is the responsibility of the kolkhozes and sovkhoses under the supervision of the veterinary service of the Ministry of Agriculture.

The comprehensive plan of measures against tularemia should be based on the landscape characteristics of the given oblast, kray, republic, both as a whole and in its various parts, as well as on the characteristics of the occupational activity of the population. The

specific characteristics of natural tularemia foci existing on the territory being serviced and the epidemiological characteristics of cases of tularemia in the past (the main types of tularemia outbreaks) are of importance. Consideration should be given to climatic, epizootological, occupational and epidemiological characteristics of the given year. In the plan which has been made out specific numerical figures should be given on each division.

The accomplishment of the comprehensive plan should be regularly checked. Checking on the accomplishment of the entire combination of measures against tularemia and rodent extermination is carried out by the executive committees of the local councils through the sanitary-epidemiological and plague-control stations.

#### Measures Directed Against Rodents and Ticks

(The technical details of the method of taking the census of and controlling rodents and ticks have been presented in appropriate instructions in the book: Tularemia. Organizational-Methodological Materials. Moscow, 1954).

The system of measures against rodents is made up of the following main divisions: 1) observation of the rodent census and timely prediction of increase in it (the forecasting service); 2) the prevention of mass rodent multiplication (agrotechnical measures); 3) measures for protection against penetration of rodents; 4) direct extermination of rodents (deratization in the broad sense).

While at the present time we have at our disposal quite a good method for carrying out the first two tasks, the destruction of rodents so far has been relatively complex, although a most important problem. It is considered that we cannot consider all rodents entirely by modern methods of deratization (V. N. Fedorov, I. I. Rogozin, B. K. Fenyuk, 1955), but we can bring about a marked reduction in their census or keep it from reaching an epizootic level. We should concentrate particularly on controlling ticks, because these arthropods are not only one of the important routes of transmission of the infectious disease but are also the reservoirs of it in nature, without the destruction of which it is difficult to count on eliminating the natural tularemia foci.

**Regular Observation of the Census of Rodents and Timely Predictions as to its Changes.** Recording the rodent census should precede measures for controlling them and should be conducted systematically, because only in this case is it possible in a timely way to predict the changes in the census and determine the volume and time not

only of extermination operations but also of other prophylactic tularemia-control measures beforehand. This work is conducted chiefly by zoologists in the departments of particularly dangerous infectious diseases of the sanitary-epidemiological station or plague-control institutions. However, in those oblasts where the zoological service is wanting in the departments of particularly dangerous infectious diseases neither the agricultural nor the public health organs are giving attention to the matter of recording the census of rodents, which advances the problem of organizing such a service.

The main attention should be given to recording the census of mouse-like rodents in the open fields, because the latter constitute reservations from which, during the autumn, these rodents migrate into and settle in inhabited places, and the range of variation in the census of rodents in these areas is very great. In every oblast a fixed observation point should be set apart where the counts are made every month. In groups of rayons which have the same landscape characteristics and which are different from the others check points should be set apart in which the investigation is made every quarter. Finally, it is advisable to keep records of the rodent census in other places twice a year, in the spring after the snow goes and in the autumn after the harvest. The data of records at the check points and at places where episodic observations are made permit clarifying the records of the fixed point as applied to areas with different landscape conditions.

The records are kept by different methods, possible for the given area, because the plan for making up the forecast is based on the results of different records. In the fields and meadows the strip method and digging up the holes in control areas are used, while in the high grass spaces the method of recording is by trap-nights. This method is suitable for recording the numbers of rodents in the woods as well as in structures. In ricks and hay-stacks the most complete data are obtained from a count made while restacking the hay; however, if it is impossible to do this the data of the records of trap-nights can be used with a certain correction. A count of the water rats is made by a combination of different methods: trap-linear, trap-area; in flood water a count of the rats per kilometer of shore line, or a count of the patches of feeding ground per 100 meters of the strip for which the census is being taken; data are also utilized on the pelts of this animal secured. The census of hares is taken by counting the tracks crossed by the control strip (after the first snow), by the difference between incoming and outgoing tracks encountered in making rounds of a certain area; the data on the number of pelts secured are also used.

By means of a comparison of the record data of the censuses

of rodents and carnivores, through comparison of the analysis of the rodent population with the farming and meteorological characteristics of the given period, the forecasting of the census is made. The most complete plan for making forecasts of numbers of small mouse-like rodents (or more accurately, the common voles) was worked out by N. P. Naumov and coauthors (1953). The prediction is given in a differentiated manner for each group of rayons having distinctive landscape characteristics. In the autumn, no later than 1 October, the main prediction is made for the winter and spring of the next year with a preliminary prognosis for the summer and autumn-winter seasons of the next year. In the spring (no later than the end of May) an improved prognosis is made, in addition, for the summer and autumn of the current year.

**Measures for Preventing Mass Occurrences of Rodents (Agrotechnical).** Taking the basic agrotechnical measures correctly and in a timely way deprives the rodents of shelter and food and is perfectly adequate for keeping the rodent census at a low level, avoiding the possibility of an epizootic. In the literature there are statements to the effect that in the fields in which there has been a breakdown of the agrotechnics the rodent census can be several times greater than in fields treated by all the rules of agrotechnics (N. A. Rashkevich, 1953). Of particularly great importance are deep fall plowing, breaking the stubble, eliminating the balks, careful and timely mechanized harvest without loss of ears or grains (Fig 78).

Pieces of straw from under the combines should promptly be carried off the fields and stacked. It is recommended that the stacks be placed on plowed-over areas at a distance from the remains of old stacks. The latter as well as the unutilized heaps of straw should be burned before the advent of the spring thaw. Scatterings of grain should be immediately picked up as much as possible, and the shoots developing from them in the spring should be mowed down. It is essential to assure a high quality of the threshing, because in substrates with the highest quantity of residual grain the rodent census reaches its maximum (I. L. Kulik, 1951). In addition, the residual grain, germinating on the surface of the stack, provides the animals living in it reserves of green food for the winter. The observance of proper crop rotation is exceedingly important. According to the data of N. A. Rashkevich (1953), in the same year the percentage of rodents caught was different in different fields of winter wheat in the same kolkhoz. In the fields where winter wheat had been present before the crop in question had been sown the rodent census was highest and amounted to 14.7 percent of the catch; on fields which had been fallow or contained crops that had been plowed under prior to the current crop of winter wheat the



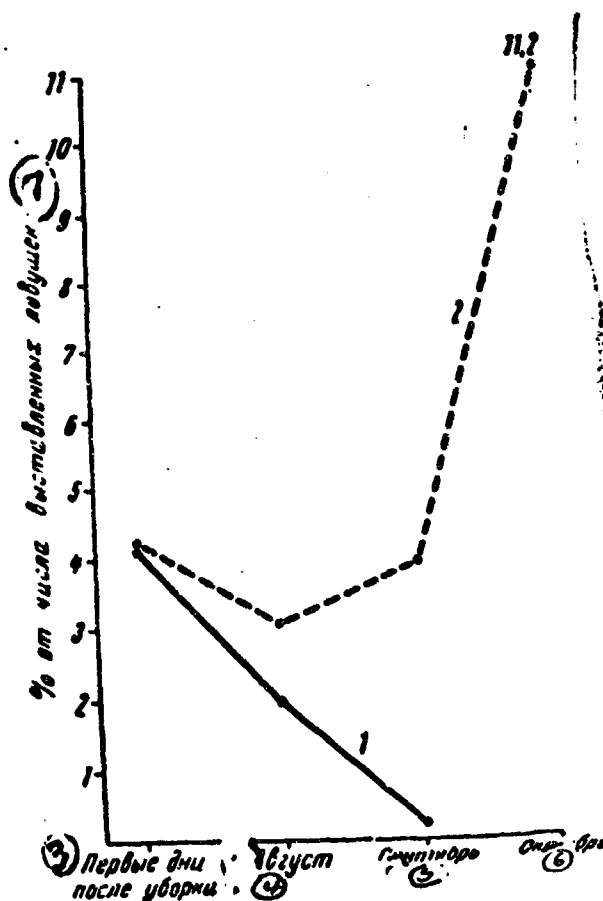


Fig 78. Change in the Population Density of Rodents (% Caught in Traps) in the Stubble of Winter Wheat (after N. A. Rashkevich). 1. In broken stubble; 2. In unbroken stubble; 3. First days after the harvest; 4. August; 5. September; 6. October; 7. Percentage of the number of traps set.

total rodents caught amounted to five-six percent. Simultaneity of the accomplishment of agrotechnical measures over large field areas has an essential influence in reducing the rodent census.

Small hillocks of grass and brush should be destroyed in the meadows, because they are shelters for the voles during the spring flood; in the autumn and winter, they are places of shelter for certain species of pasture ticks (for example, *Dermacentor pictus*). In addition, the small hillocks of grass and brush interfere with the mechanization of haymaking operations. Before the ripening of the seeds (in the middle of the summer) the weeds should be cut, with particular

care, for a distance of one kilometer around inhabited places, in edges of fields and forest strips. The latter should be cleared of brushwood, because the mature cluttered forest strips are good areas for the survival of rodents (N. V. Bashenina and V. V. Kucheruk, 1952; N. A. Rashkevich, 1953). This goal is achieved by cleaning up gardens, mowing the grass on them and using row-spacing.

For the purpose of preventing an increase in the rodent census in inhabited places sanitary order should be maintained on their territory: destroying weeds on the streets and in the areas around the farmsteads; filling in unnecessary ditches and the elimination of broken-down structures unnecessary for farming, regular cleaning of rubbish, straw and farming wastes in yards, streets, and vacant areas; setting up cesspools and refuse pits in every yard (in the cities these are to be made of concrete or lined with metal), bringing the reserves of straw, hay a distance of 10 meters away from living quarters; not permitting that hay and straw be placed on the roofs of houses. In carting hay and straw from the field to inhabited places they should be shaken out again before being loaded on the cart.

Measures for Protection Against Rodent Penetration. These measures are of particularly great importance in inhabited places, because it has been determined that without them it is impossible to reduce the rodent census in various structures and installations for a long time by extermination operations alone. It is most difficult to ratproof various structures; this is of secondary significance for the prophylaxis of tularemia and more important for the prophylaxis of other infectious diseases (plague, leptospirosis, rat-borne rickettsial disease, etc.). However, other species of rodents can subsequently use the portals of entry of the rats. It is also difficult to bar access to mice. It is simplest to protect the various structures against penetration of voles.

Ratproofing of the building is assured by a stone foundation one meter in depth, by concrete floors in the cellars, packing in the holes of pipes and tubes with cement, screening the cellar windows and ventilation openings with strong fine metal screens, lining the doors of the first two floors with metal to a height of 30 centimeters. All objects attached to the walls of the buildings should be removed so that rodents cannot climb up on them. Storehouse premises should be constructed either on a stone foundation (with a stone base 80 centimeters in height and asphalted or concrete floors) or should be set on wooden or brick columns 80 centimeters tall. Iron deflectors are driven into the wooden columns, while ropes dipped once a month in a mixture containing one part of a phenol-cresol-coal-tar-oil mixture and 200 parts of some lubricant (waste oil, pitch, petroleum distillation residues [mazout]) are wound around the stone columns. Lean-to ladders

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should be taken down from such storehouses immediately after use. Walls and ceilings in the storehouses should not be made hollow within, because they serve as nesting places for rodents. Goods within the storehouse should be set at a certain distance from the walls on shelves or platforms raised 85-90 centimeters above the floor. Iron deflectors are driven into the pedestals of the platforms and shelving. For the purpose of protecting storehouses located directly on the ground it is recommended that they be surrounded with a strip of chloride of lime three-five centimeters thick and 20-25 centimeters in width, sprinkling half the quantity of the substance twice a month (B. V. Voskresenskiy, 1943). However, the effectiveness of this method needs additional checking.

Particularly important structures should be surrounded with gutter traps. The gutters recommended for this purpose 40-50 centimeters in depth protect only against common voles, and then not always, as has been shown by experiments (P. A. Sviridenko, 1950). For protection against mice it is necessary to make gutters 70 centimeters deep, 50 centimeters wide at the top and 70 centimeters wide at the bottom. At the bottom of such a gutter poisoned bait is set in order to accelerate the death of rodents which fall in there and prevent undermining of the walls. With the latter aim in view it is also recommended that the outer wall of the gutter be made sloping so that the rodents can leave easily (V. V. Modestov, 1925).

It is advisable to dig such gutters only in solid ground and they should be cleaned and fixed regularly. This complicates their use for such abundant and widely scattered objects as ricks and haystacks, which frequently, incidentally, are located on soft arable soil, where gutters with inclined walls will constantly cave in. In such cases it is best to plow under a strip 10 meters in width around the haystacks.

Measures for Rodent Extermination. Destruction of rodents can give a perceptible effect only in combination with other measures: agrotechnical, sanitary, rodent-protective. For the purpose of controlling rodents various methods are used: chemical, mechanical, biological, and a variety of the latter -- the bacteriological method. Methods of destroying rodents have been presented in detail in the collection Obligatory Measures for the Control of Mouse-Like Rodents (1950) and in appropriate instructions. (Tularemia. Organizational-Methodological Materials, pages 141-168). Here, the characteristics of extermination of only those species of rodents which have a bearing on the spread of tularemia are being discussed briefly.

Mechanical Methods of Extermination. This is one of the oldest and most common methods. Because of its safety for man and domestic animals it is available for the population at large. It is used

chiefly in inhabited places in addition to the chemical method, and in some installations (food, children's institutions) it is used almost exclusively. The most catching tools are traps of the "Hero", "Hero Trap" types [ordinary mousetraps], wire traps of various kinds, animal traps, and, for the larger rodents, the Nos 0 and 1 leg-holding traps. Extensive use is also made of various "continuous-action" traps. T. N. Baranovskaya (1956) successfully used a sticky mass made of rosin and castor oil (2:1) for the purpose of catching rodents; this mass is applied to sheets of tarpaper, plywood or cardboard. These sheets, placed in the rodent holes, act without drying for 15 days, and in four-five days all the rodents living in the holes are caught on them. In addition, among the mechanical methods of rodent extermination mention should be made of catching them with gutter traps (see above) with subsequent regular destruction; elimination of rodents at the time of re-stacking haystacks and threshing ricks, and, finally, the method of complete digging-up of the holes proposed by N. I. Kalabukhov (1944). This method, effective in limited areas where there is a small number of rodents, is not very well suited for mass deratization because of its laboriousness.

**Chemical Methods of Extermination.** At the present time, these are the main and most effective methods. Poisoned baits, poisoned drink, dusting the holes with poisons, etc. are used. Recently, attempts have been made to use carbon monoxide (exhaust gas of a gas-generator automobile) for the purpose of destroying rodents in vegetable vaults, ricks, haystacks, piles and even holes (Stavropol' Plague-Control Institute, 1953; N. M. Dukel'skaya and S. V. Vishnyakov, 1953; N. V. Nekipelov and N. D. Altareva, 1954). Before beginning the use of the gas the object being treated should be well sealed off or covered with canvas.

Of the toxic chemicals very extensive use is made of the preparation "krysid" (alpha-naphthylthiourea, the "ANTU" of foreign authors). The high degree of selective toxicity of the preparation for rodents makes it possible to use it in smaller concentrations than other poisons, which makes it practically harmless for man and animals. Because of the insolubility of the preparation in water and its low wettability it can be used not only for bait but also for poison water suspensions and for dusting holes. All this has assured the successful mass application of "krysid", including its use in such institutions as food and children's institutions.

Another toxic chemical widely used at the present time is zinc phosphide. Like "krysid", it is insoluble in water but readily dissolves in gastric juice, where, after combining with hydrochloric acid, it produces phosphine, which is toxic to the organism. The preparation

itself attracts rodents and bait containing it is eagerly eaten. A defect of this substance is its toxicity for man and animals, which makes it necessary to observe precautionary measures in using it. Apart from these preparations use is made (less often) of warfarin, sodium fluoride, sodium fluosilicate, barium carbonate, sodium arsenite, red squill, and others.

As bait for mice, bread, groats, dough, cooked vegetables and grain (sunflower seeds are particularly attractive) are used. For voles it is better to use vegetable green baits made of minced fresh grass or steamed hay. Water rats prefer bait made of fresh carrots or the root portions of water plants. The poisoned bait is prepared either by mechanical mixing of the food product and the poison or by wetting or digesting the grain bait in the solution of poison or by sticking powdery poisons to the bait. For the purpose of sticking the poison to the grain use is made of vegetable oil, flour or starch paste; for the purpose of making poison stick to grass, water is used to which a small amount of molasses has been added. For the purpose of destroying the mice the following concentrations of toxic chemicals are needed in the baits: krysid, one percent; zinc phosphide, six-10 percent; warfarin, four-five percent; barium carbonate, 10 percent; sodium fluoride, 1.5-two percent. Sodium arsenite is used in a five percent solution for the purpose of wetting grain. According to the data of N. N. Bakeyev and coauthors (1956), rye and wheat grains require a smaller concentration of zinc phosphide (six percent), whereas for the hull grains of oats a concentration of the poison of 10 percent is needed, because when the rodents remove the hulls of the oats part of the poison is removed with them. The total consumption of bait varies from 200 grams to two kilograms per hectare depending on the rodent census.

Ready-made baits are distributed either on the surface in places which are most visited by rodents or in the rodent holes or in special bait boxes containing openings for the entrance and exit of rodents (P. A. Sviridenko, 1951). N. V. Bashenina and V. V. Kucheruk (1952) suggested scattering baits (in the woods, strips of forest) under heaps of dry branches or straw (long-term effect points).

Attempts have been made to mechanize the laborious process of scattering bait. The use of a seed drill for this purpose has not justified itself because of the excessive expenditure of bait (about three kilograms per hectare). L. A. Surkova and Ya. V. Tsys (1956) used a tractor drill with an attached trailer on which there were seats for six workers who scattered the bait by hand. On perennial grasses solid sowing was used; a spoonful of the bait was thrown out every five seconds. This gave an expenditure of 1.2 kilograms of bait per hectare. On winter crops the bait was scattered only around the colonies

of voles encountered, which considerably reduced its outlay. Where the width of the trailer is 40 meters and the speed of the tractor is five-eight kilometers an hour the area treated per worker per shift was increased to 30 hectares (in the case of sowing by hand it was three-seven hectares); the fuel consumption amounted to 520 grams per hectare. V. A. Abramov and A. S. Musatova (1956) used continuous sowing of the bait from a moving truck: two workers scattered the bait from the sides of the truck and one from the back; together they covered a strip 30 meters in width. Despite the somewhat greater expenditure of bait than in the case of tractor sowing (1600 grams per hectare) the area worked per worker per shift was increased to 100 hectares (the truck moved at a rate of 12-15 kilometers per hour), while the fuel consumption per hectare was reduced to 200 grams. All this reduced the cost of the work from 57 kopecks per hectare for manual operations to 27 kopecks per hectare. In the case of high rodent densities (5,000-10,000 holes per hectare), where this method is particularly expedient, the outlay of bait should be increased to three kilograms per hectare simultaneously with increase in the concentration of the toxic chemical.

Recently, it has been determined that for deratization purposes water with krysid or zinc phosphide applied to its surface can be used. In the wintertime, in unheated storehouses snow which has been dusted with poison can be scattered as bait (V. I. Vashkov, 1952). N. P. Naumov and coauthors (1951) suggested a method of dusting the holes with the poisons for the purpose of controlling the common voles, which eat baits poorly. He based this idea on the fact that the rodents, going over the dusted areas, get poison on their fur and paws and are poisoned by it when they lick it off. Where there are a small number of hole entrances a hand duster is used in the form of a rubber bulb with a tip, while in the treatment of very much infested areas it is best to use a knapsack sprayer and duster with a long tip in order to be able to work without bending over. For each entrance hole it is necessary to use from 0.5 to one gram of krysid or zinc phosphide. Recently, recourse has been had to dusting the holes in various installations and structures as well as dusting artificially-created holes, bait boxes, vegetation surrounding the holes, etc.

**Biological Methods of Extermination.** Underlying them are the natural interrelationships of rodents and their enemies -- animals, birds and pathogenic microbes. It has been determined that carnivorous animals (foxes, polecats, weasels) and predatory birds can to a considerable degree destroy a rodent population in various areas (S. S. Folitarek, 1948; Yu. A. Myasnikov and coauthors, 1953). For this reason, hunting four-legged carnivores which exterminate rodents is forbidden (white polecat, weasel) or is restricted (fox, raccoon-like

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dog, steppe polecat). For the purpose of attracting predatory birds to the fields (buzzard, merlin, common kestrel, and members of the corvine family) it is recommended that poles 2.5 meters in height with a transverse bar at the top be set at distances of 100 meters from one another and the birds readily sit on these. Attracting birds by means of setting up these poles is particularly effective in perennial grass fields which have an increased rodent census. With extermination of the rodents the poles are moved.

In inhabited places more extensive use should be made of dogs and cats for controlling rodents. In spite of the opinion which has been created in the literature to the effect that cats when infected die of tularemia and can serve as sources of infection for people (B. V. Voskresenskiy, 1943; A. F. Komarova, 1945; K. A. Dorofeyev, 1951, and others), they are not very susceptible and in practice they are insensitive to tularemia (T. N. Dunayeva, 1954). However, in occasional cases cats should be considered mechanical vectors of the infection on their teeth, claws, etc. after they bite, scratch and others. These cases should not serve as an obstacle to extensive utilization of cats for destroying rodents, including rodents in foci of tularemia; simply caution should be shown with respect to handling these animals. An interesting report by T. Ye. Boldyrev (1955) on the use of cats during the Second World War for destroying mice in dugouts, blindages, and trenches in foci of tularemia states there were no authenticated cases of infection of people with tularemia from cats observed, despite the intensive use of them (more than 2,000 animals). Apparently, death of the cats when masses of rodents appear occurs from other infectious diseases which are transmitted to the cats by the rodents and are lethal to the cats. Possibly, cats die of listerellosis; in the winter, this disease is widespread among rodents. The cats may also die of infectious diseases of virus origin.

**Bacterial Method of Extermination.** The paratyphoid microbes of Danysz, Merezhkovskiy, and Isachenko, pathogenic for rodents but safe for man and agricultural animals, are used for rodent control. However, for precautionary purposes it is not permitted to use this method in food and children's institutions nor in animal husbandry farms where there are young animals. Biscuits or ground grain are dipped into the culture of microbes grown on a special medium or else the microbe culture is mixed with flour to form a dough. For the purpose of controlling mice on the premises the bait is distributed in 2.5 gram units in the holes or in bait boxes. In the fields the bait is dispersed according to the calculation of five grams per eight-10 entrances to holes; in the haystacks it is placed in bait boxes or is distributed in large pieces (no less than the size of a hen's egg) calcu-

lating one gram per cubic meter of the substrate.

In the presence of high rodent densities the salmonella epizootic which occurs can spread by contact through the excretions of sick rodents and from animals' eating their bodies. There are indications of the fact that 60-90 percent of the rodents can die of an epizootic and it terminates after two-three weeks. Part of the rodents acquires immunity to the microbes, as the result of which it is recommended the deratization be completed with chemical agents. This method is applicable only when there is an increased rodent census; in the case of low rodent densities there will not be contact between the rodents, and, therefore, there will be no conditions for the spread of the epizootic. The use of deratization cultures is advisable only at moderate temperatures; high temperatures as well as alternate freezing and thawing rapidly cause their deaths. The effect of the bacterial bait is increased considerably if 0.05 percent krysid is added to it.

**Characteristics of the Control of Small Mouse-Like Rodents in the Field.** In the fields, the main measure which prevents mass multiplication of small mouse-like rodents, is the observance of agrotechnical rules (see above) and direct rodent extermination is used only as an auxiliary measure. For a long time it was believed that the extermination of mouse-like rodents in the field should be conducted only when they begin to cause notable damage, that is, when their census reaches threatening dimensions. One of the last proponents of this conception was N. I. Kalabukhov (1944). In the past the error and inefficiency of such control was pointed out by A. N. Formozov (1937), B. K. Fenyuk (1937) and N. P. Naumov (1946). They suggested a better system of control: the daily performance of extermination operations in places of accumulation of rodents (survival areas) in the spring when the census of the animals is minimal and it is possible to destroy the potential progeny. It has been determined that the survival areas are usually small (10-12 percent) compared with all the fields, which accelerates the work and makes it less expensive. This system of measures was developed in detail by N. P. Naumov (1946) and it was checked by him and coauthors (1951) under field conditions. In the spring, as soon as the snow melts all the survival areas of the rodents are determined by means of investigation: the edges of the forest, strips of the forest, cultures of perennial grasses, waste land, balks and lapses, slopes of gullies and ravines, shoulders of roads and ditches, weeds, residues of ricks and haystacks. The latter, as far as possible, should be burned promptly. Immediately after this in the survival areas all the holes found are baited. Before the beginning of vegetation, when the rodents feel a shortage of food, poisoned bait can be used; later, it is preferable to dust the holes. In both cases it is best to use krysid



which guarantees safety of the work. If it is difficult to find the holes (strips of forest, edges of the forest, lapses) the bait can be placed under artificial bait heaps made of weeds or straw. Such heaps with a long-term effect and containing grain bait should be set up for the winter, checking them once a month. During the summer a regular check is made of the various lands and fresh holes found are baited by means of dusting with krysid (simultaneously with the investigation). The taking of such a combination of measures reduces the census of mouse-like rodents (voles) to negligible figures through the autumn inclusive (Fig 79).

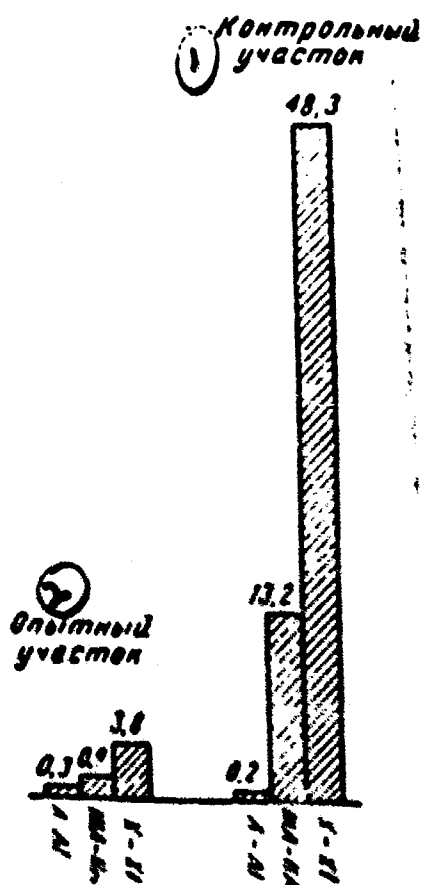


Fig 79. The Number of Voles According to Record Data of the Census in 1949 in Experimental and Control Areas (on the Average per Hectare) (after N. P. Naumov, N. M. Dukel'skaya and V. V. Dombrovskiy, 1951). 1. Control area; 2. Experimental area.

Straw and haystacks, etc. are placed on the plowed-over areas, and in the autumn-winter the rodents are exterminated in them by means of placing boxes containing poisoned bait in the straw according

to the calculation of one box per 10 linear meters of the perimeter of the object. The sizes of the boxes are 40x10x10 centimeters. Instead of the bait a straw cutting or chaff dusted with poison can be put into the box. The bait should be renewed and the dusting should be repeated every month. All these operations are conducted by the well-instructed kolkhoz rat eliminators and specially designated workers of the sovkhoses under the supervision of an agronomist. The advantage of this schema lies in the fact that the main extermination measures do not coincide with the period of mass agricultural operations. For the purpose of estimating the quality of the operations carried out before and after the treatment records of the rodent censuses are made. The work is considered satisfactory if the census comes to less than 30 percent of the original after the baiting.

The rodent census can be maintained permanently at a low level only through regular yearly accomplishment of this combination of measures.

**Characteristics of Control of Mouse-Like Rodents in Inhabited Places.** The main conditions for successful rodent control in inhabited places is a good sanitary status of their territory and construction (see above). It is also desirable to ratproof the latter.

Rat-extermination measures are taken in inhabited places by special organizations of the Ministry of Health, Ministry of Grain Products and other departments (chemical, bacteriological, mechanical methods). The operations are conducted according to a plan made out previously in accordance with agreements reached and requests made. In addition, the disinfection stations or disinfection departments of sanitary-epidemiological stations are obliged to carry out continuous deratization of the entire inhabited place no less than twice a year on the basis of the decision made by the local Council of Workers' Deputies. The most convenient times for this are the periods of mass migration of rodents into the inhabited places: the period of advent of the cold weather (October-November), and the period of the spring thaw (March-April). This is done in combination with simultaneous participation of institutions of various departments engaged in rodent extermination. Attention should also be given to the destruction of rodents in areas of private home owners near farmsteads. Simultaneously with regular planned deratization the directors of enterprises, institutions, and home owners should through their own efforts carry out regular concurrent rodent extermination by mechanical and biological methods.

In conducting rodent extermination in inhabited places by chemical and bacteriological methods the established safety regulations should be observed; home owners should be notified of these by correspondence. Rodents killed by the mechanical method should be given

over to the laboratory of the department of particularly dangerous infectious diseases of the sanitary-epidemiological station (or to the plague-control station) for bacteriological study; in the absence of proximity of these they should be burned.

**Characteristics of Rodent Control on Transport.** Control of mouse-like rodents on transport is of particularly great importance, because infected rodents can be carried from foci of tularemia epizootic along with the freight, which has been noted repeatedly in practice. On the right of way of railroads chiefly the same general sanitary and rodent-control measures should be taken as on the territory of inhabited places. The supervisors of the various objects and areas of the railroad service are responsible for accomplishing these measures. Rodent-extermination measures are conducted by the disinfection departments of the railroad sanitary-epidemiological stations according to contract. In the work of continuous deratization in inhabited places adjacent to the railroad the railroad disinfection service should participate, providing for measures in its own installations and right of way in accordance with the total comprehensive plan. In the right of way weeds should be destroyed, and mouse-holes should be baited.

On water transport the main attention should be given to control of rodents on the territories of ports, pier structures, as well as on the territory of water-transport workers' settlements. Special detachments of reservoir and port sanitary-epidemiological stations accomplish this by chemical, bacteriological and mechanical methods. For the deratization of ships the gas method is used (when rodents are found on the ships). It is forbidden to transport goods which show signs definitely indicating that rodents had settled there (gnawing, excretions, bodies of rodents).

**Characteristics of Control of the Water Rat.** Despite the fact that the water rat is one of the main sources of tularemia over the major portion of the territory of the USSR and does considerable damage to the national economy measures for controlling it have been worked out inadequately to date. The following meadows improvement operations may be recommended against the water rat: drying meadow bogs, hill cutting, mowing sedge and cutting reeds. The boggy sections should be cleared of brushwood and old stumps, where the water rat finds shelter. The main method of exterminating the water rat so far is mechanical. This is chiefly a mass hunt for it with the aim of securing the pelts. The rat is caught on objects protruding from the water during the spring flood as well as by leg-holding traps in other seasons of the year. For the purpose of improving this method of controlling the water rat a good price has been set on its pelt.

S. S. Folitarek and coauthors (1951) suggested setting traps

along specially plowed furrows at a distance of 10-15 meters from one another; the water rat catch is increased by four times thereby with half the expenditure of time. The plowed furrows are imitations of the trails well-trodden by rodents, which the animals use for their movements, for which reason the water rats run along them readily. In the spring, as soon as the ground dries, furrows 22-23 centimeters in width and 15-20 centimeters in depth are plowed. They are arranged around the water body (bogs, lake "zaymishcha" [this is a Siberian name for lowland bogs covered over with reeds and rushes]). The furrow should have smooth perpendicular walls and a horizontal level bottom. In setting and rebaiting the traps one should walk along the furrow, which packs its bottom down and prevents the formation of pathways which distract the rats from the furrow. Another mechanical method of exterminating the water rat by the same authors is that of catching the animals with iron or Ruberoid cylinders dug into the plowed furrow; these cylinders have wooden bottoms 40 centimeters in depth and a diameter of 16-22 centimeters. This is less successful, because it is laborious work to dig the pits for these cylinders every 10-15 meters and aside from this the outlay of large quantities of roofing materials is not profitable. S. S. Folitarek's suggestion that the water rats be caught in pits sprinkling a kilogram of chloramine in each, is entirely impractical.

For the purpose of exterminating the water rat Yershova and Gulidov have successfully used baits made of pureed potatoes, carrots and dry potatoes containing five percent zinc phosphide or calcium arsenite, placing them on feeding-ground areas, and at the time of a flood on small floats anchored to the bottom which move freely upward and downward along a pole with the water level (B. Yu. Fal'kenshteyn and B. S. Vinogradov).

S. S. Folitarek, A. A. Maksimov and others dispersed poisoned bait made of pieces of white (under-water) parts of sedge and cattails five-10 centimeters in length on plow furrows every 10-15 meters. The bait (one kilogram) was mixed with 50 grams of zinc phosphide, or was soaked for two days in three percent sodium arsenite solution. As the bait was eaten and dried out it was replaced.

V. V. Kucheruk and coauthors (1958), checking different poisoned baits and different methods of using them, established the fact that water rats eat baits containing zinc phosphide best of all; those containing krysid three times less; and even less for the other poisons. The rats find the bait most completely and quickly along the water's edge; and it should be set out here. They take the bait somewhat later within the limits of the border of the sedge on the shore, while outside of this border they practically do not find the bait. In the spring and in

the summer the rats take best to baits made of delicatated under-water parts of the sedge, cattail and young stems of the water thistle. Early in the spring and in the autumn the animals readily eat bait made of carrots, by the use of which containing two percent zinc phosphide it is possible to exterminate almost completely the water rat population in various water bodies. Grain bait at the same time gave a much poorer effect, in contrast to the data of M. G. Yakovlev and coauthors (1955) who recommended grain bait. The lack of promise of these baits for control of the water rat has been shown convincingly also by V. S. Yedykina (1956) in a series of laboratory and field experiments. Control of the water rat is conducted with the same personnel as for control of the mouse-like rodents.

**Methods of Exterminating Ixodial Ticks.** Control of ixodial ticks under natural conditions still offers difficulties. It can be conducted along different lines: 1) agrotechnical measures; 2) destruction of rodents, the hosts of the larval and nymph stages of the tick (see above); 3) the use of natural enemies of the tick; 4) extermination of ticks on domestic cattle; 5) extermination of ticks in nature.

The combination of these measures makes it possible successfully to control chiefly the pasture ticks, particularly those species which in the mature phase are parasitic chiefly on domestic animals. As far as the rodent hole ixodial ticks are concerned, which in all phases of development are parasitic on rodents and other small mammals, control of them is difficult. Destruction of these ticks is possible only by means of exterminating their hosts or dusting the holes.

In veterinary practice long ago a regular change of pastures was suggested as a measure for controlling tick infestation of cattle. However, considering that various species of ticks can live without food for up to five years and others have a biological life cycle which drags on for several years, stopping the grazing of cattle on a pasture for even several years may not achieve its purpose. Particularly useless is stopping the use of the pasture for a total of a year (A. L. Dul'kin, 1952). After grazing on an area infested with ticks it is not recommended that cattle be driven again on areas free of ticks. Substitution of artificially sown pastures for the natural tick-infested pastures is more advisable. Even pastures which have sown for many years (five years) contain 10-30 times fewer ticks than natural pastures (A. V. Fedyushin, 1949). Pastures of annual crops, in which the ticks die out completely during the plowing, can be considered practically free of parasites. In case it is necessary to use natural meadows as pastures they should be freed of brush and hills which constitute shelters for the hosts of the larval and nymph phases of the ticks.

An attempt has been made to use a small hymenopterid,

*Hunterellus hookeri*, imported from the United States, for controlling ticks; this is capable of parasitizing the nymphs of many species of ticks, but this attempt ended without success (N. I. Alfeyev and Ya. V. Klimas, 1958). This species of insect as well as another parasite species were then found to be natural inhabitants of certain places of the USSR (G. S. Pervomayskiy, 1947; D. I. Blagoveshchenskiy, 1948; M. N. Nikol'skaya, 1950). However, further development of tick control by means of using these parasites did not occur, apparently because of the difficulty in breeding them.

Chemical methods of controlling ticks have been more successful. First arsenicals were used for this purpose, treating tick-infested cattle with them (Ye. N. Pavlovskiy, 1928, 1935; N. O. Olenov and B. I. Pomerantsev, 1948; N. I. Alfeyev, 1935, and others). Then, for the same purpose the safer preparations DDT and hexachlorane became popular (I. A. Yegorov, 1948, 1949; V. I. Kurchatov and coauthors, 1951; Ye. I. Pokrovskaya, 1953, and others). Specifically, these preparations were used with full success for destroying ticks, the vectors of tularemia (L. A. Pogodina and N. G. Olsuf'yev, 1950; V. G. Petrov, 1958). According to the data of L. A. Pogodina and N. G. Olsuf'yev (1950), most effective for destroying *Dermacentor pictus* ticks was 10 percent DDT dust; treatment with this four or five times at seven-day intervals was fully adequate for protecting cattle against ticks (Fig 80). Fifty grams of the dust are used for a single treatment of a single cow or unshorn sheep; after the cows shed and the sheep are shorn the expenditure is reduced to 25-30 grams. Five percent hexachlorane dust and a five percent ointment of these preparations in vaseline (25 grams per animal) was effective. It is recommended that the ointment be applied to places in which the dust is poorly retained. A poorer effect is given by aqueous suspension. Only the favorite spots of the ticks are treated (neck, withers, ears and upper part of the head); the dust is applied with a gauze pledget; the ointment, with the hands in rubber gloves. A defect of hexachlorane is that the odor of this preparation can be smelled in milk of the treated animals.

Aside from vaseline, vaseline oil (V. G. Petrov, 1958), lubricant grease and solar oil (V. I. Kurchatov, 1951; Ya. S. Bolgov and Ye. I. Pokrovskaya, 1952, and others) have been proposed as solvents for DDT and hexachlorane. However, the last-mentioned substance in a number of cases causes burns of the skin in the animal. N. Ye. Yegorov and F. M. Leont'yev (1949) suggested a one percent hexachlorane solution in gasoline, using it in combination with seven percent dust. B. V. Paychadze (1951) recommends dissolving DDT and hexachlorane in creolin (in a proportion of 1:2 and 1:5, respectively), and then preparing 2.5 percent aqueous emulsions from the solution.

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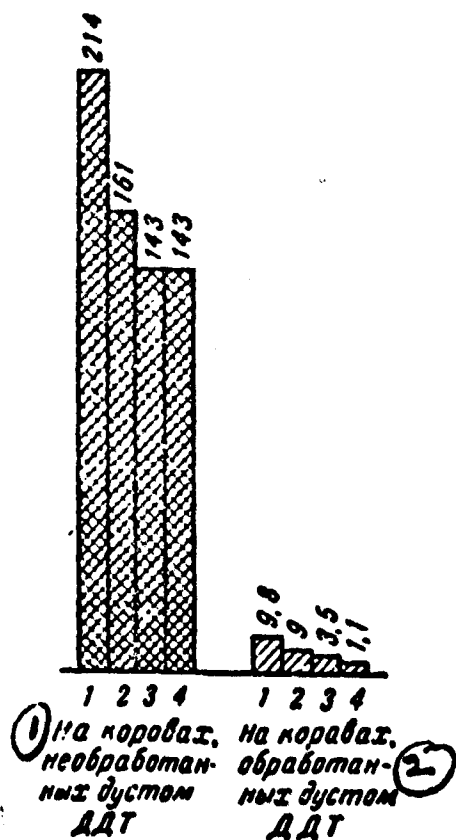


Fig 80. Average Census of *Dermacentor pictus* Ticks on a Single Cow Depending on Treatment with 10 Percent DDT Dust. On the left, in four kolkhozes where the cattle were not treated with DDT dust; on the right, in four kolkhozes where the cattle were treated with DDT dust (after L. A. Pogodina and N. G. Olsuf'yev, 1950). 1. On cows untreated with DDT dust; 2. On cows treated with DDT dust.

However, according to the data of Ya. S. Bolgov and Ye. I. Pokrovskaya (1952) they are less effective than the oily solutions. According to the data of N. V. Matikashvili and A. P. Rostomashvili (1955) a solution of hexachlorane in creolin causes irritation of the animal's skin. These authors replaced creolin with an aqueous suspension of a cellulose sulfate extract (a byproduct of paper production) with the addition of askangel clay [bentonite from Askania, Georgia, USSR] for stability of the suspension. Such a hexachlorane suspension does not cause irritation of the skin and exerts an acaricidal effect. Nabokov's sticks have been used successfully also for tick control in cattle (K. P.

Andreyev and A. M. Mitrofanov, 1955).

Recently, in the control of ticks which are vectors of taiga encephalitis methods of tick extermination have been worked out in nature by means of dusting the vegetation with DDT or hexachlorane (N. N. Gorchakovskaya and coauthors, 1953, and others). A single treatment of the vegetation up to one meter in height with these preparations (one gram per square meter) led to the complete destruction of *Ixodes persulcatus* and *Dermacentor marginatus* ticks (Fig 81).

Further observations have shown that a single ground treatment of forest litter with DDT dust using 30-50 kilograms per hectare assures the extermination of all phases of development of *Ixodes persulcatus* ticks during the year of treatment and the maintenance of this effect for four years (N. N. Gorchakovskaya and coauthors, 1958). Hexachlorane treatment gave poorer results. The use of an airplane for dusting the vegetation made it possible to conduct extermination operations over considerable areas. M. V. Davydova (1958) reported the successful experiment of exterminating *Dermacentor marginatus* ticks and *Rhipicephalus rossicus* ticks on the territory of a gully by means of ground treatment of the vegetation with 12 percent hexachlorane dust.

#### System of Prophylactic and Antiepidemic Measures for Cases (Outbreaks) of Tularemia

The first work on the epidemiology of tularemia in the USSR contains very few indications of prophylactic and antiepidemic measures taken. First of all recommendations were worked out for the prophylaxis of the occupational outbreaks of tularemia, since this type of case was predominant during the initial years of study of tularemia. For the prophylaxis of other types of tularemia outbreaks and control of them initially agents were recommended which were used for the prevention of such infectious diseases as plague (continuous de-ratization, the wearing of masks, protective goggles, protective clothing and others), intestinal infections (prophylaxis of water-and food-borne outbreaks) or malaria (prophylaxis of arthropod-borne outbreaks).

Subsequent antiepidemic practice showed that the specificity of the tularemia infection requires making considerable corrections and additions to these methods already known. Particularly great experience in the prophylaxis of tularemia was accumulated during the years of the Second World War, because on territories liberated from temporary occupation considerable tularemia outbreaks occurred the elimination of which was carried out by the public health organs (V. N. Apekhtin, 1945). In solving these practical problems the low degree



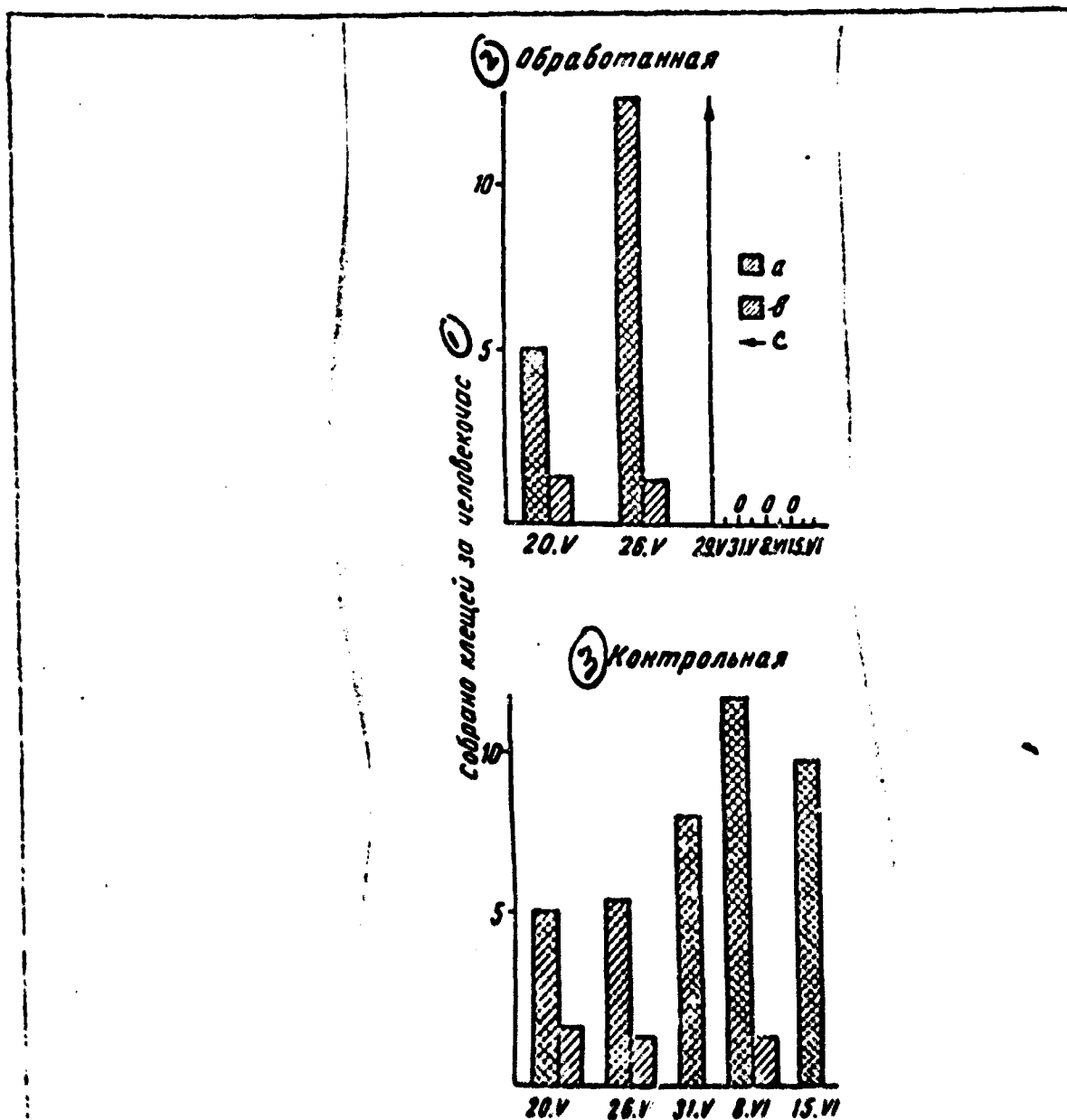


Fig 81. Tick Infestation of Control and DDT-Treated Area (after N. N. Gorchakovskaya and coauthors, 1953). a. *Ixodes persulcatus*; b. *Dermacentor marginatus*; c. Day of treatment of the area with the preparation. 1. Ticks collected per man-hours; 2. Treated; 3. Control.

of suitability or poor effectiveness of a number of measures previously proposed was clarified and a search for new more effective prophylactic measures became necessary. At the present time, for every epidemiological variety of tularemia a combination of the basic measures has been worked out making it possible not only to reduce and completely

eliminate the disease but, in a number of cases, even to prevent its occurrence, despite the presence of an epizootic. The majority of these measures has been approved in appropriate instructions of the Ministry of Health USSR.

We consider it useful to begin this part of the work with the measures taken for the prophylaxis of tularemia in any case and then we shall proceed with the system of measures for the various types of tularemia. The sequence of this presentation is the same as that in Chapter VI.

#### Measures Common to all the Epidemiological Types of Tularemia

Among these measures mention should be made chiefly of immunization with living tularemia vaccine with the aim of creating a maximum immune segment among the population which may be threatened with tularemia. Vaccination has been described in detail in Chapter X; in the presentation of the prophylactic methods for various types of disease (outbreaks) which follows only the characteristic features of its application in every individual case will be pointed out. Among the general measures mention should also be made of the extermination of rodents, which are mass sources of tularemia. In the presentation which follows we shall indicate only in a most general form the characteristics of rodent control with the aim of prophylaxis of various types of outbreaks, because methods of rodent extermination have been described in greater detail above.

Thorough acquaintance with the clinical aspects, diagnosis, epidemiology and prophylaxis of tularemia by district medical workers is also a very important general measure. This is accomplished at brief seminars for medical workers (two-day seminars for physicians; one-day seminars for medium-level medical workers). They are carried out under the direction of an epidemiologist and specialist on infectious diseases who have taken a three-five-day seminar at the oblast sanitary-epidemiological station or plague-control station. A good knowledge of tularemia by district workers considerably improves the diagnosis and detection of tularemia patients. According to our data (Yu. A. Myasnikov, 1955), in the region in which only sporadic cases of tularemia were recorded medical workers were poorly acquainted with this infectious disease, and the number of patients with tularemia who went undetected per every case recorded exceeded the same ratio in another ratio by a few-score times; in the other rayon, outbreaks of tularemia were noted constantly, and the local medical workers were well acquainted with it.

With increase in the rodent census an attempt should be

made to detect the epizootic before the occurrence of cases among people for the purpose of timely development of prophylactic measures. "The practice of 'signaling' the presence of tularemia after detection of cases in people should be decisively censured" (V. N. Ter-Vartanov and coauthors, 1943). In detecting the tularemia epizootic its boundaries need to be determined, for which purpose an extensive questioning of the population is organized concerning the presence of deaths of rodents, while around the place where the epizootic is detected rodents are used for bacteriological examination with the investigator moving from the place at which the epizootic is found toward the periphery. Sources and transmission factors of the infectious disease suspected should be confirmed bacteriologically (see Chapter VIII).

When an epizootic or cases of tularemia are detected in a rayon mass house-to-house rounds should be made with the aim of detecting patients with tularemia. For this purpose the sanitary authorities and active members of the Red Cross who have first been instructed by a medical worker should be used. In the case of a tularemia epizootic encompassing large territories simultaneously, which can lead to the occurrence of disease in various inhabited places, the detection of all febrile patients through the forces of medical workers alone is practically impossible.

Hospitalization of the patient with tularemia is essential, despite the fact that he does not constitute a source of infection to those around and can be hospitalized in general internal medical wards. Under hospital conditions laboratory methods of diagnosis can be used most completely and the patient can be given effective therapy, while timely laboratory diagnosis of tularemia, according to the data of L. M. Khatenever (1943) is the basis of the control and prophylaxis of this infectious disease. Many cases have been described where a tularemia outbreak was diagnosed as "influenza", "malaria", etc. for a long time (Ya. S. Kon', 1947, and others), as the result of which there was a delay in taking appropriate prophylactic measures.

A considerable segment of the population artificially immune to tularemia makes the tularin test unsuitable as the only diagnostic test. Combined use of the tularin test and the complete agglutination test with checking of the increase in titer (see Chapter VIII) is a reliable method of laboratory diagnosis of tularemia. This latter reaction, along with the Widal, Weil-Felix, and Wright tests, should be performed in every clinical laboratory, and if there is none in the rural hospital the blood (or serum) should be sent to the rayon or oblast laboratory. (In an extreme case one cc of serum dried on clean white paper can be sent). When tularemia is present in the region of natural foci all patients admitted to the hospital who have had a fever for more

than a week, including patients with sulfonamide and penicillin-resistant pneumonias as well as all patients who have lymphadenitis, should be examined serologically for this infectious disease.

Hospitalization is necessary even at the climax of the outbreak, when the diagnosis of tularemia has already been established. In this case hospitalization pursues the aim of excluding other acute infectious diseases (typhoid and typhus, malaria) along with internal medical aids to the patient, because patients with these infectious diseases have frequently been taken as patients with tularemia at the climax of the tularemia outbreak. Also onlays of typhus fever on tularemia have been described (N. S. Polyanskiy, 1947, and others). Only after laboratory confirmation of the diagnosis of tularemia and the exclusion of other infectious diseases can the patient be discharged for outpatient treatment with a satisfactory course of the disease. Note should also be made of the compulsoriness of sending out a special report on each case of tularemia, which is frequently forgotten by physicians.

Immediately on receiving the special report the sanitary-epidemiological station (or sanitary-epidemiological department of the hospital) should make a careful epidemiological study according to a chart which has been specially worked out (the use of an epidemiological examination chart for the patient with zoonoses and arthropod-borne diseases is permitted). Epidemiological investigation of the first cases in an inhabited place should be made by a physician, because only the physician can properly establish the clinical-epidemiological parallels and determine the epidemiological type of disease, which is particularly important for the proper selection of the combination of anti-epidemic measures.

A very essential part in the prophylaxis of all tularemia outbreaks is played by sanitation education work among the population, and in some types (domestic infection, infection through the water of open water bodies and others) this becomes a leading factor. The higher the degree of sanitary culture of the population with respect to tularemia the fewer cases of infection there will be. When a tularemia epizootic occurs in a region the sanitation propaganda about the prophylaxis of this infection should be intensified considerably and should be conducted by all available agencies: the distribution of popular brochures, leaflets, placards, inclusion of articles in the local newspapers, demonstration of popular movies, lectures over the radio at enterprises and institutions, schools and kolkhozes before movie audiences, individual and group talks with the population in house-to-house rounds and at the time of vaccination. In accordance with the type of disease there is a change only in the groups of persons on the sanitation education of whom the main emphasis is put.

## Characteristics of Measures for Different Epidemiological Types of Tularemia

**Measures for an Arthropod-Borne Infection Through Blood-Sucking Diptera.** Infection through blood-sucking Diptera -- mosquitoes and horseflies -- is closely connected with the water rat, because this animal is usually the source of infection of the insects. In some places, apparently, the infection of Diptera from sick hares is possible. Despite the fact that this type of tularemia is among the oldest in Russia and was one of the first to be observed (in 1877 near Astrakhan'; in 1921, in West Siberia, etc.), prophylactic measures for arthropod-borne outbreaks for a long time remained very little developed. Various authors believed that the measures for the arthropod-borne outbreaks are identical with those against malaria (B. N. Voskresenskiy, 1943, and others), but this opinion should be considered without basis. Other authors erroneously recommended measures used for agricultural outbreaks -- agrotechnical measures, destruction of waste straw, early threshing, prohibition of the carting of untreated grain and other food products (V. G. Beletskiy, 1948; A. L. Kartasheva, 1950) -- for the prophylaxis of arthropod-borne outbreaks. There is no need to prove the unsuitability of these measures for the prophylaxis of arthropod-borne infections.

At the present time, the main method of prophylaxis of cases of the arthropod-borne type is planned vaccination and revaccination of the entire population (beginning with the age of seven) living in an area of the soddy-alluvial-boggy foci of tularemia. Persons, including citizens who have temporarily come into the area of these foci for summer work (haymaking, care of orchards, improvement measures, etc.) or for fishing, hunting, etc., should also be vaccinated. With the occurrence of cases of tularemia as the result of arthropod-borne infection, which indicates the presence of non-immune persons among the population, first of all the non-inoculated population should be forbidden to go into areas affected by the epizootic (river valley, shore region of a lake, etc.) temporarily before complete vaccination is carried out. The population should be permitted to enter the infected territory after the results of inoculation have been checked (in the presence of a positive reaction), that is, on the 15th day after vaccination. This comprehensive method of prophylaxis is particularly effective when large population groups are simultaneously exposed to the possibility of infection on a limited area.

Using the imposition of a quarantine on infected meadows in combination with mass one-stage vaccination, we obtained the oppor-

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tunity of eliminating completely a considerable arthropod-borne outbreak in Permskaya Oblast in a very short time. Of great significance in these prophylactic measures was a well-organized mass sanitation-education operation among the population which facilitated both the observance of a quarantine and the giving of the inoculations. If in the focus mass inoculations have been given before permission to enter the affected areas is given under the control of the tularin test, which considerably shortens the time for which the territory needs to be isolated.

Because blood-sucking Diptera can transmit other infectious diseases aside from tularemia and, in addition, they can give considerable trouble to people those inoculated against tularemia should observe a number of additional measures during work in a river valley. The windows and doors of field camp quarters and inhabited places located along the edges of the river valley should be screened, and if it is impossible to screen them bed nets should be used during sleep. The inside of the houses, tents and field transport is carefully treated with DDT. If there is a very low vermin census the areas of skin should be left exposed as little as possible and use should also be made of Ye. N. Pavlovskiy's headnets. The net is attached to the headgear so that it protects the neck and falls on the shoulders, leaving the face exposed. The protective action of the net is from five to 10 days. Dipping the nets in dimethylphthalate and dibutylphthalate jelly prolongs and improves their repellent effect.

Recently, for individual protection of man against vermin various agents have been recommended. Most effective is dimethyl ester of phthalic acid, dimethylphthalate. The preparation can be applied to the clothing or to the exposed skin, on which it is rubbed with a cotton pledget or with the palms. For the purpose of a single coverage of the skin of the arms and legs 60 drops (three grams) of the preparation are required; for the purpose of covering the skin of the trunk up to the waist -- approximately the same amount. Treated clothing preserves its repellent effect under conditions of the North up to five days. The repellent effect of treated skin is briefer, because during work the preparation is moistened with perspiration and its effect depends on the temperature, humidity and other factors. In a temperate climate this effect lasts four-five hours (F. Yu. Rachinskiy and co-authors, 1951); in a hot steppe area, one-four hours (V. A. Nabo'ov, 1955). Dimethylphthalate is effective against all groups of dipteran vectors with the exception of horseflies when they attack en masse.

Recently, the daytime shelters of blood-sucking Diptera -- grass and bushes -- have been treated successfully with DDT and hexachlorane preparations (P. G. Sergiyev and coauthors, 1953). Insects flying into the treated territory die or lose the ability to bite. Only

territory on which people work or live should be treated, and the barrier around it should be 30-50 meters in width; 0.4-0.5 gram of the commercial preparation is used per square meter of surface. In the case of treatment from the air it is better to use aviation spraying than aviation dusting, because the liquid wave is less carried away and requires a lower outlay of the preparation. It is also recommended that aerosols obtained from burning hexachlorane smokepots of the NBK (G-17) type be used for the control of winged blood-sucking Diptera in an open locality. The best results are obtained from the use of smokepots during the hours of dawn in windless weather. The residual effect of the smoke lasts for 10-12 days with an expenditure of 0.8-1.0 gram of commercial hexachlorane per square meter of surface (V. A. Nabokov, 1955).

Because water rats chiefly are the sources of infection in arthropod-borne outbreaks, and work in the river valley takes place in close contact with sources of water a combination of measures should be taken, aside from measures for the prophylaxis of arthropod-borne infections, for the prophylaxis of water-borne infections associated with an epizootic among water rats (see below).

Measures for Arthropod-Borne Infection from Pasture Ticks. In the Soviet Union infection of people with tularemia from the bites of ticks or as the result of crushing them is noted comparatively rarely, by contrast with the United States; nevertheless, measures should be taken with respect to this route of transmission of the infectious disease also. Cases of infection from ticks undoubtedly occur more often than they are diagnosed, and, in addition, the fact should be taken into consideration that in addition to tularemia ticks can be vectors of a number of other infectious diseases dangerous to man (tick-borne encephalitis, hemorrhagic fevers, tick-borne rickettsial diseases and others).

The main measure is vaccination of the population living in the locality in which cases of attachment of ticks associated with tularemia have been noted (or may be suspected). Measures of personal prophylaxis, which have been well developed and are finding extensive application for the prophylaxis of tick-borne encephalitis (G. S. Pervomayskiy, 1946), tick-borne rickettsial diseases, etc. may be additional methods used. These measures consist of wearing protective clothing (V. A. Eskin and coauthors, 1944), which prevents ticks from crawling on the body, and regular checkups with the removal of ticks which have attached themselves at the time of visiting habitats infested with the tick vectors of tularemia (forest, steppe, bushes, etc.). For the purpose of increasing the effect the protective clothing is treated with tick-repellent substances, for example, dimethylphthalate (S. G. Gladkikh and Ye. D. Chigirik, 1954). Camp should be made and tents

should be pitched in areas cleared of grass and litter.

When ticks are removed mechanically from cattle and when sheep are shorn caution should be observed that the ticks be not crushed. After contact with ticks (or their excretions) it is essential to wash the hands with soap carefully. In order to avoid carrying the ticks into the living quarters dogs should not be permitted to come into them; dogs collect a large number of ticks on themselves; also, it is not recommended that dry twigs just brought from the forest be used as fuel in the stoves. Among the prophylactic measures for this type of disease mention should also be made of extermination of ticks in nature and on domestic cattle, which has been discussed in greater detail above.

Measures for Prevention of Infection in Hunting and Securing the Pelts of the Water Rat, Muskrat and other Animals. In this method of infection cases of tularemia can occur among hunters, those skinning the animals, as well as among workers in hide-storage places. Frequently, the water rat occupation takes on a grand scale and a considerable part of the population of villages located near rivers or lakes participate in it. The main prophylactic measure for these outbreaks is preliminary vaccination of all persons participating in the occupation and members of their families against tularemia. According to existing instructions on the prophylaxis of occupational tularemia infections (Tularemia. Organizational-Methodological Materials, pages 169-173) the groups mentioned are vaccinated before the beginning of the hunting season (no later than 15 days before), but after the preliminary performance of a tularin test for the purpose of checking immunity. Only persons in whom the tularin test has been found to be negative are inoculated. After the results of the inoculation are checked in all persons in whom the vaccine has been inoculated a certificate is issued giving them the right to participate in hunting water rats, muskrats, hamsters, moles and other animals and in securing their pelts. Measures of personal prophylaxis recommended previously -- cotton-gauze respirators, protective goggles, rubber gloves, bactericidal ointments for smearing the hands -- are not used at the present time; however, general sanitary measures preventing dissemination of the infection have maintained their significance completely.

Hunting and the securing of pelts should be conducted in an organized manner (with special hunting brigades) which facilitates the inspection of observance of sanitary regulations. All catchers and skinners should be provided with special overall suits, rubber or oil-cloth aprons, waterproof boots, and oversleeve gloves. The hair should be covered with headgear. Killed water rats are carried in iron pails or covered boxes or wooden boxes lined with iron inside. After they have been emptied these objects as well as the transport on which



the catch has been carried should be treated with three percent lysol solution or three percent carbolic acid or two-three percent clarified solution of chloride of lime.

The catching of sick animals (inactive) and collection of their bodies for the removal of pelts is prohibited. It is recommended that the bodies found and sick animals which have been killed be sent for bacteriological examination or that they should be burned on the spot. Cases of mass deaths of water rats, muskrats and other rodents should be immediately reported to the local public health organs, and the latter should promptly inform the department of particularly dangerous infectious diseases of the sanitary-epidemiological station or plague-control station about this. There is no particular reason for not permitting the removal of pelts from animals in which "abscesses", "fistulas", or "scars" have been found. Tularemia in water rats has a course like an acute septic process and rapidly ends in death (T. N. Dunayeva, 1954), as the result of which the abscesses and fistulas and particularly the scars do not manage to develop. Subcutaneous glands and excretions from them are often taken for these lesions.

Removal of the pelts and drying them at home is categorically forbidden. For these purposes skinning stations should be organized. They are set up in barns, huts, etc. no nearer than one kilometer to an inhabited place and sources of water supply in a place agreed upon by the sanitary organizations. At the skinning station it is essential to have chloride of lime, five percent carbolic acid solution or three percent lysol solution as well as shovels for burying the carcasses. The latter are thrown into a pit 1.5-two meters in depth, and each batch is sprinkled with chloride of lime. When the pit has been filled to a level one meter from the level of the soil it is covered over with earth and a new one is dug. It is forbidden to feed the water rat carcasses to domestic animals and utilization of them for commercial purposes can be permitted only with the permission and under the control of organs of sanitation. On completion of this work the hunters and skinning station workers should wash their hands and special suits (boots, apron and oversleeves) with disinfectant solution, remove them, take off their special overalls and outer clothing, and carefully wash their hands and faces with soap.

The dried pelts are brought to the storage point in a waterproof container. Extensive sanitation education work should be developed among workers in this occupation and among those at the storage point. When there is a tularemia epizootic in this region the pelts are kept in a dry warm room for two months before being shipped from the storehouse of the regional storage office, which guarantees the death of the pathogen (A. N. Knyazevskiy and V. A. Bordnikov, 1930; P. V.

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Somov and coauthors, 1939). If immediate shipment of the pelts is required they should be treated in a special chamber with chlorpicrin calculating 50 cc of the preparation per cubic meter of chamber with a load of 300 water rat pelts per cubic meter or 200 muskrat pelts per cubic meter. Treatment is carried out at a temperature of 30-35° and exposure time of 20-24 hours (P. V. Somov and coauthors, 1939) with subsequent airing of the pelts in the chamber for two-five hours and two-three days in the storeroom until the complete disappearance of the lacrimatory effect of chlorpicrin. Treated and untreated pelts are put in different storehouses. Checking on the disinfection of the pelts is carried out by organs of the veterinary-sanitation inspection, which issue a certificate of disinfection for every batch of pelts being sent out.

In the case of occurrence of a tularemia epizootic among water rats the hunt should not be stopped. Conversely, it should be intensified, because it contributes to reducing the census of this rodent and stopping the epizootic. It is characteristic that this circumstance was emphasized by even the first investigators of occupational outbreaks (A. Ya. Krol', 1933; A. A. Miller, 1937), but various subsequent authors have neglected this rule and recommended that the occupation be stopped (A. L. Kartasheva, 1950, and others), and only recently has the expediency of this measure been proved and encouraging prices for the water rat pelts been introduced. N. G. Olsuf'yev and coauthors (1958) note that on the territory of the Volga-Akhtubinsk River Valley hunting the water rat has played an important part in the prophylaxis of tularemia in recent years, holding back the increase in the water rat census and the development of tularemia epizootics among them.

Of chief significance in the prophylaxis of tularemia in the muskrat occupation is the extermination of water rats and regulation of the muskrat census. Vaccination of muskrats with living vaccine recommended by certain authors (S. S. Folitarek and others) is calculated more to soothe muskrat breeders than to create a pack of muskrats resistant to tularemia, because the vaccine strain cannot be transmitted among the animals by the contact or alimentary route. Persons participating in the hunting of such animals highly sensitive to tularemia as moles and hamsters in regions enzootic for tularemia should also be inoculated against tularemia and should observe the same sanitary regulations as in the water rat occupation.

Hunting and storing pelts of animals susceptible but not very sensitive to tularemia (souseliks, beavers, black and brown rats) does not present a real danger of infection of people with tularemia, because the bacterial seeding of the bodies of these animals is relatively

low (T. N. Dunayeva, 1954). According to the evidence of R. V. Konyshev (1934) in the North Caucasus hundreds of thousands of pelts of sousliks were secured; however, no cases of tularemia were noted among persons participating in this occupation.

In connection with this, prophylactic measures for the occupation in which these species are involved should be directed at prevention of other infectious diseases which may be transmitted from them to man. However, some authors still insist on vaccination of persons occupied in hunting sousliks against tularemia (N. I. Makarov and coauthors, 1955).

Measures against Infection during the Hunt of Hares and Consumption of Their Meat as Food. Measures in these methods of infection should be considered simultaneously, because isolated alimentary infections from hares are encountered extremely rarely, and in the great majority of cases they are combined with contact infections from the skinning of the animals and eviscerating the carcasses.

As the main prophylactic measure compulsory vaccination should be recommended for all hunters as well as their family members through the local hunting societies. The latter is particularly essential, because alimentary infection from inadequately cooked hare meat is very severe and at times fatal because of the massive nature of the infective dose. Before the beginning of the hunting season sanitation-education work should be conducted with the hunters. It is essential that hunters report the occurrence of death among hares and bring the bodies of the hares for examination to the sanitary-epidemiological station.

After hunting hares, skinning the animals or eviscerating the killed hare as well as after preparing hare meat as food it is necessary to wash the hands with soap carefully, and existing cuts and abrasions on the skin should be smeared with iodine. Children should be eliminated completely from those operations associated with the treatment of the killed hare.

The consumption of the meat of hares with a markedly enlarged spleen or with necrotic nodules on it or on the liver should be avoided. It is particularly important to boil or roast hare meat carefully in small pieces, whereby the former method is more reliable for disinfection of it. All objects used for removing the pelt and working with the carcass should be boiled or treated with dry heat for a half-hour. If the hare has been caught in the season in which ticks are parasitic measures should be taken to prevent their crawling away: the killed hare should be placed in a tightly-tied bag; before skinning the bag and the body of the hare should be inspected (particularly carefully around the ears and on the head), and all crawling and attached ticks

should be removed and destroyed. The pelt should not be left in a house for the purpose of drying.

When a tularemia epizootic is found among hares the sale of hare carcasses on the market should be temporarily forbidden. It is desirable that this restriction be made also when an epizootic occurs among small mouse-like rodents, with which hares, particularly gray hares [*Lepus europaeus*] are in close contact in the winter. At meat control stations bacterioscopy should be performed of smears taken from the spleens of hares and the precipitation test should be performed with an extract of the organs. If there is a positive test for tularemia the carcasses should be removed from consumption.

Measures against Infection from the Water of Open Water Bodies. Prophylaxis of infection from water of open water bodies is more difficult than prophylaxis of well outbreaks. Water not only of ponds, small lakes or brooks but sometimes also of rivulets is infected (S. P. Karpov and V. M. Popov, 1949), in connection with which it may be consumed simultaneously by the inhabitants of many inhabited places without any difference in age. In contrast to well outbreaks the water of open water bodies can be infected simultaneously in many places from water rats or muskrats or their bodies infected with tularemia. This infection of the water can occur, according to the observations of A. A. Selezneva (1949), continuously for more than seven months, but even this time apparently is not the maximum. According to the data of A. A. Selezneva (1949, 1950), water-dwelling animals are of additional significance in infection of water of brooks; however, according to the data of N. G. Olsuf'yev and coauthors (1959) as well as of American investigators the participation of them is not obligatory (see Chapter V).

The difficulty of chlorinating water of open water bodies, particularly running water, complicates the use of this method of prophylaxis in this case. The main measure for the type of infection under analysis is planned vaccination of the entire population living along the shores of water bodies exposed to infection (for example, in natural foci of the foothill-brook type, whereby in cases in which there is a particular danger inoculations should be given even to children beginning with the age of two. Of great importance is extensive sanitation-education work with warning about the necessity of using only boiled water for drinking, washing and laundering. V. P. Bozhenko (1950) in the region of foci of the foothill-brook type recommends constructing good wells in the villages and field camps for water supply of the population. Along the shores of water bodies water rats should be killed by means of organization of an active hunt; the reeds, sedge and rushes should be carefully cut, which deprives the rats of shelter from carnivores.

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The entrance of infected water of water bodies into the main water supply system can cause simultaneous infection of a large number of people (M. I. Tsareva, 1945, and others).

Such cases have been observed from consumption of the water of commercial water mains for drinking purposes. Prohibiting this as well as removing connections between drinking and commercial water supply mains are important prophylactic measures. When infection is found from the tap water the water supply should be stopped immediately. The use of water from the drinking water supply main is renewed only after careful chlorination of it. M. I. Tsareva (1945) succeeded in stopping new cases by bringing the concentration of residual chlorine up to one milligram per liter. Ts. N. Gotovskaya and M. Ye. Magaram (1945) proved experimentally that for the purpose of greater reliability in the disinfection of water from the tularemia pathogen use should be made of a dose of active chlorine equal to 1.5-two milligrams per liter. It should be noted that the entrance of house mice sick with tularemia into elevated tanks and reservoirs can imitate infection of open water bodies with tularemia microbe. Therefore, with the occurrence of such outbreaks it is necessary to inspect these reservoirs for the presence of dead bodies of rodents and immediately remove them if they are found, after which the water should be chlorinated.

**Measures against Infection from the Water of Infected Wells.**  
Cases of tularemia in people from the consumption of well water most frequently arise in the winter in connection with an epizootic among small mouse-like rodents and only occasionally come from the falling of sick water rats into the well. After the determination of the infected source, which is usually done without any particular difficulty on the basis of questioning the patients, the well is immediately covered and sealed, stopping the utilization of water from it until it is disinfected.

It is desirable to take a sample of water promptly and send it to the department of particularly dangerous infectious diseases of the sanitary-epidemiological station for bacteriological examination. Purification and chlorination of the well should be begun without delay.

This is accomplished in the following way. The volume of water is determined in the well, for which purpose the area of the surface of the water is multiplied by the depth of the water layer. Then, in order to make further operations safe, the water is hyperchlorinated until the appearance of a sharp odor of chlorine in it (80 grams of chloride of lime per cubic meter of water). Eight hours after this the water is pumped out of the well entirely, the bottom and the walls are cleaned of silt and washed with one percent chloride of lime solution.

If on cleaning the infected well bodies of rodents are found they should also be brought for bacteriological examination to the department of particularly dangerous infectious diseases. The mud and silt taken out of the well are buried at a depth of 0.5 meter and a distance of no less than 20 meters from the well, downstream with regard to the movement of ground water. After repair, cleaning the well and filling it with water disinfection is accomplished again by means of adding 40 grams of chloride of lime per cubic meter of water and exposing this for six hours. After treatment the water is pumped out until the chlorine odor in the water disappears, and then use of the well is permitted (V. I. Vashkov, 1952).

For radical prophylaxis against infections of this kind careful and everyday inspection of the sanitary status of wells are necessary. The walls of the wells should be elevated above the surface of the ground by no less than 80 centimeters and should not have any cracks making penetration of rodents possible. Around the upper, underground portion of the well a "lock" should be made 0.5 meter in width and 1.5-two meters in depth made of well kneaded loam which has been tamped down in layers. In the winter, when a coating of ice forms the ice should be placed around the well in order to prevent the entrance of rodents into it. The wells should be supplied with a tightly-fitting cover and a bucket for general use. When a tularemia epizootic occurs in the inhabited place it is necessary from time to time to chlorinate the wells for prophylactic purposes. During this period only boiled or chlorinated water can be used for drinking or washing.

Measures against Infection at Winter Agricultural Operations. In the recent past (before 1952) the great majority of agricultural tularemia infections in the USSR occurred during the winter threshing, as the result of which timely completion of the threshing of the grain (no later than October) was considered the most important prophylactic measure for agricultural outbreaks. However, as was justifiably noted by N. G. Olsuf'yev and coauthors (1950), "timely threshing cannot completely prevent cases among people if the rodent census is high". At the present time, the so-called "threshing" outbreaks of tularemia have almost stopped, which is associated with the extensive incorporation of mechanization into harvesting operations, but cases of infection are still noted associated with other types of agricultural operations (sorting and drying of the grain, preparation of seeds for sowing, carting potato clumps, straw and hay to animal husbandry farms, etc.). According to our observations, in Tul'skaya Oblast in 1949, in the complete absence of unthreshed ricks the cases of infection during agricultural operations amounted to 13.5 percent of all cases of tularemia.

In foci of agricultural outbreaks of tularemia all the rural population beginning with school age should be inoculated. The high degree of epidemiological effectiveness of this measure for the prophylaxis of agricultural infections has been noted by many authors (see Chapter X). The inoculations should be given to the city population which has been sent out for agricultural work in a locality unfavorable with respect to tularemia. In accordance with the instructions of the Ministry of Health USSR (Tularemia. Organizational-Methodological Materials, page 180), in cases where threshing is necessary in the winter and spring in the presence of mass deaths of mice (epizootics) in the ricks only those vaccinated against tularemia or people who have had it and react positively to the tularin test should be permitted to work.

Before the introduction of vaccination many authors recommended that the threshing and other agricultural operations be conducted in respirators, protective goggles, etc. At the present time, these measures can have only general hygienic but not antiepidemic significance. The recommendation of A. A. Miller (1937), B. V. Voskresenskiy (1943) and others that agricultural operations be carried out in rubber gloves as a measure for preventing tularemia was unacceptable in practice. However, in carrying out agricultural operations in foci of tularemia it is necessary to observe measures of personal hygiene -- to work in canvas oversleeve gloves, to carefully clean the dust off the clothes (or change the clothing), wash the hands with water and soap, etc. before eating or after the conclusion of the work.

In those cases where not all persons participating in the agricultural work have been included in the inoculations and cases of tularemia have appeared this work should be stopped immediately for a time, and all the workers should be investigated with the tularin test. (Persons who have had tularemia in the past or who have been inoculated, which is learned by means of questioning, are temporarily assigned to the operations which cannot be stopped (for example, carting and giving of feed to the cattle), but simultaneously a tularin test is performed on them also). Forty-eight hours after checking the results of the test only persons who show a positive reaction to the tularin test are permitted to work at the infected installations; the others are vaccinated and permitted to work at these installations no sooner than 12-14 days later, when there is a positive skin test to the inoculation. At one installation, despite the previous high morbidity rate, we succeeded in preventing new infections completely, after threshing of the cereal crops had been stopped for a total of two days.

In foci of agricultural outbreaks measures for the prevention of the straw, grain and other agricultural products or for the dis-

infection of them are more difficult. These measures have been described in detail in the appropriate instructions (Tularemia. Organizational-Methodological Materials, pages 180-183); here, we shall dwell only on the most important factors. The basis of prophylaxis consists of not permitting the settlement of small mouse-like rodents (common voles, house mice, etc.) in the stacks of straw, haystacks, etc. left on the fields or barnyards for the winter or the prevention of settlement of rodents in storehouse premises where the gathered grain is kept. The stacks of hay and straw should be entrenched in gutter traps, which prevent the settlement of rodents, but this measure is not applicable to freshly plowed fields. In these cases it is useful to plow out a strip no less than 10 meters in width around the haystacks and straw stacks. New stacks should not be placed near the remains of the straw left for the winter. The latter should be burned before the spring thaw.

For the purpose of destroying rodents in the stacks of straw or hay in various cases the objects were subjected to gas from chlorpicrin. However, this measure is unacceptable, because chlorpicrin makes straw unsuitable for use and deprives the seeds of oil-bearing crops of their germinating power (N. I. Kalabukhov, 1944), whereas the bactericidal effect of it is doubtful under these conditions. B. V. Voskresenskiy (1940) reports the isolation of the tularemia pathogen on examination of straw two weeks after exposing a straw stack to chlorpicrin gas. The use of cyanide cakes and cyanide dust is inadvisable in the straw stacks because of their volatility (N. I. Kalabukhov, 1944). Apparently, the use of exhaust gas of an automobile is more practical, but thereby no bactericidal effect is produced.

With the aim of disinfecting the infected object it is recommended that it be restacked and that the lower layers of straw in which the rodents are concentrated be burned. However, this method is laborious and it is not much used in practice. Grain and straw from infected objects are carefully freed of bodies and rodents. The grain is put in storehouses into which the rodents have no access and is permitted for use after it is kept for 30 days after the evident of persistently positive temperatures (above 10°).

In the presence of a temperature which is constantly below zero the tularemia pathogen is preserved in grain and straw no less than 192 days (L. A. Pomanskaya, 1957). The recommended quarantine periods change at different temperatures (Table 42).

As an additional measure which does not eliminate the need for quarantining the grain airing of it can be used in a layer of 30-40 centimeters with mixing, which contributes to more rapid and uniform heating of it. The solar irradiation for 24 hours recommended



Table 42

Time of Natural Disinfection of Grain and Forage Infected by the Tularemia Pathogen at Different Maintenance Temperatures and the Recommended Quarantine Periods (after L. A. Pomanskaya, 1957)

① Температура воздуха	② Предельный срок сохране- ния возбу- дителя, наблю- давшийся в опытах (дни)	③ Температура воздуха	④ Рекоменду- емый срок карантина (дни)
8—12°	56	8—14°	60
15—25°	35	15—20°	40
20—30°	19	21—25°	35
		26—30°	20

1. Air temperature; 2. Maximum time pathogen can be preserved in experiments (days); 3. Air temperature; 4. Recommended quarantine period (days).

by the instructions does not achieve its purpose at low temperatures (L. A. Pomanskaya, 1957). Food and fodder grain are disinfected by heating at a temperature of 70° for 30 minutes (L. A. Pomanskaya, 1957). Seed grain is sprinkled with a solution of formalin, 1:90 (for the commercial formalin) with an expenditure of 40-50 liters of the solution per ton of grain and it is kept under canvas for two hours but no more, with subsequent airing of the grain, spread out in a fine layer for 24 hours. For garden seeds a lower concentration of formalin solution is acceptable -- 1:150 (L. A. Pomanskaya, 1957). On the certificate a note should be made about the treatment given. After re-stacking and after they are freed of the bodies of rodents the straw and hay are kept for the same periods as grain before consumption (Fig 82). Carting of hay and straw out of regions which are unfavorable with respect to tularemia is permitted only when the epizootic stops, after it has been freed of the bodies of rodents and after it has been kept for the periods indicated above exclusively in the pressed form in the absence of injury to the bales by rodents. Permission for carting is given by the veterinary inspection organs with the consent of the local sanitary-epidemiological or plague-control stations.

Measures against Infection at Home. These cases occur from the mass migration of small mouse-like rodents (house mice, common voles and others) into inhabited places and the importation of

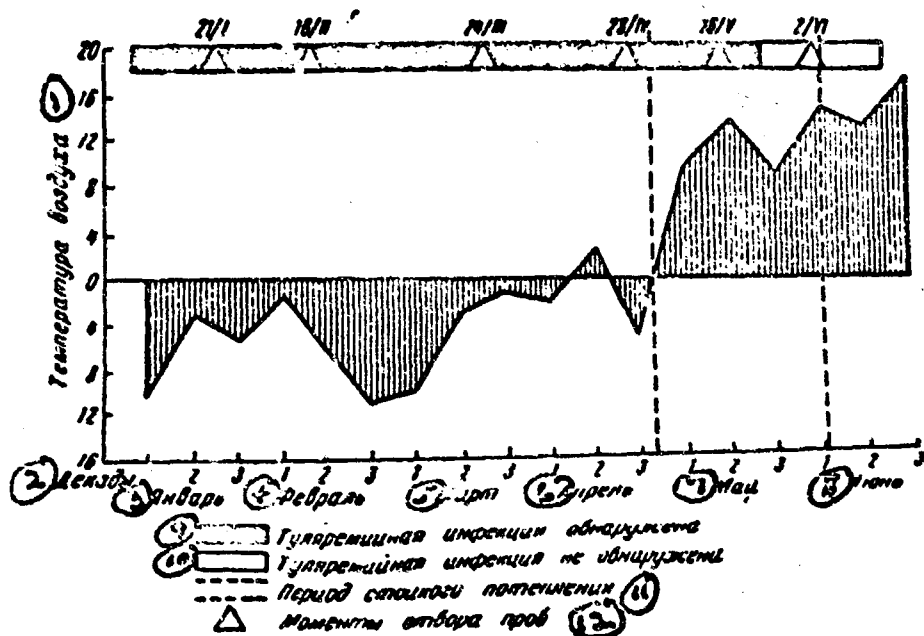


Fig 82. Time of Disinfection of Residues of Rye Straw Stacks Under Natural Conditions (after L. A. Pomanskaya). 1. Air temperature; 2. 10-day periods; 3. January; 4. February; 5. March; 6. April; 7. May; 8. June; 9. Tularemia found; 10. Tularemia not found; 11. Period in which it becomes persistently warm; 12. Times of taking samples.

the epizootic there. In the South they are noted even in October-November; in more northern oblasts, in December-February. In the northern oblasts a new wave of increased morbidity is observed during the period of the spring thaw in March-April. In connection with the possibility of extensive spread of a tularemia epizootic among small mouse-like rodents over the territory the most effective prophylactic measures against domestic infections are complete vaccination of the population (beginning with the age of two in cases where there is a special danger) and complete, repeated deratization in the inhabited places. The latter is accomplished by the disinfection service of the public health organs (chemical and particularly bacteriological methods) as well as by the population itself (mechanical traps of various kinds). In the case of a danger of occurrence of domestic outbreaks more extensive use should be made of cats, which successfully protect houses against rodent penetration.

In the prophylaxis of domestic cases of tularemia exceptionally great attention should be given to cleaning the territory of the inhabited place, chiefly the territory directly adjacent to houses and various buildings, cleaning up heaps of rubbish, straw, weeds, because these places, being intermediate habitat areas during the migration of rodents from the fields, contribute to the settlement of the houses and buildings by mice and voles. For prophylactic purposes when new construction is erected it should be made ratproof and in houses which are already erected all rodent holes which appear should be filled in regularly. An appropriate sanitary status of the houses should be maintained also. Of particular significance is careful protection of food and water supplies existing in the house against rodents. Hay and straw which have just been brought in from stacks in the field should not be permitted to be used. L. M. Khatenever and I. N. Mayskiy (1946) recommend that wet disinfection with the usual disinfectants be used in premises dirtied by mouse excretions in the presence of epidemic indications. In any case, in the presence of mouse droppings on the floor wet cleaning [mopping] should be carried out in order to avoid raising dust. The bodies and excretions of rodents found should be burned or buried in the ground without touching them with the bare hands. On finding food products contaminated by rodents in the house they should be carefully cooked or thoroughly roasted and those products with which this cannot be done should be destroyed.

Because domestic cases of tularemia can appear in the form of sporadic cases of the disease along with epidemic outbreaks as well as because of the fact that in this type of infection the thoracic and abdominal forms of tularemia occur most frequently and these are difficult to diagnose, timely detection of patients assumes importance. In one of the first "mouse" outbreaks described by V. A. Bernikov (1934) a considerable number of patients with tularemia went under the diagnosis of "epidemic influenza", and only 40 patients with the bubonic form of tularemia were detected. Other authors also note that in the case of a domestic infection of a considerable number of patients a diagnosis of influenza, typhoid fever, or other diseases is made. In connection with this febrile patients should be detected in regular house-to-house rounds and should be hospitalized for the purpose of clarifying the diagnosis. For the purpose of prophylaxis of infection at home mass sanitation-education work among the population assumes importance.

Measures against Infection from Food Products Infected in the Storehouse or Public Dining Room. In this case all cases of disease usually have a single common transmission factor -- a food product infected at the storehouse, which is readily detected on questioning

the patients; therefore, elimination of diseases of this kind offers no great difficulties. Cases stop appearing immediately after disinfection or destruction of the infected food product.

For the prophylaxis of group cases coming from food products an investigation should be made regularly of the sanitary status of food enterprises, dining rooms, restaurants, bread factories and bakeries, food stores, food storehouses and markets. Special attention should be given to assuring the ratproofing of a food installation and the maintenance of food products under conditions in which they cannot be accessible to rodents. All rodent holes in the premises should be sealed with cement or clay containing scrap glass or should be stopped up with iron. Food products should be kept in a tightly-sealed container or on shelves away from the walls; on the pedestals of these shelves iron deflectors should be driven in which are expanded below. At all food installations regular deratization is accomplished. Aside from planned rat extermination operations conducted according to agreements with the disinfection service, in every storehouse, store, dining room rodents should be exterminated daily through the forces of the regular personnel working there, using traps. The traps should be set regularly, and the rodents caught should be burned or buried in the ground. Before releasing the food products they should be carefully checked with the aim of detecting spoilage and contamination of the food by rodents, and those in which excretions, bodies or the gnawing of mice are found should be immediately made unavailable for consumption.

The portions of the food product as well as the bodies, excretions and gnawings should be sent to the nearest department of particularly dangerous infectious diseases for bacteriological examination. Food products which have been gnawed on or dirtied by rodents should be regarded as suspicious of tularemia infection regardless of the results of the bacteriological examination. It is forbidden to use them without preliminary processing. First of all, they should be freed of the bodies and excretions of rodents. Pieces containing gnawing marks are cut around or removed, after which the food product is carefully loiled, thoroughly roasted or dried in a drying oven at a temperature of 70° for no less than an hour. Those products which cannot be subjected to thermal processing should be destroyed.

It should be taken into consideration that mice can contaminate food products not only with the tularemia pathogen but also with the pathogens of paratyphoid fever, listerellosis and other infectious diseases. The statement that food which has been on ice for a long time is free of tularemia (Review of British Military Medicine, Vol I, 1942) is not true. Through the work of V. M. Tumanskiy (1937) and other Soviet authors the long preservation of the pathogen in frozen food

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products has been proved.

The result of a smooth-running food sanitation inspection in the Soviet Union is the rare occurrence of cases of tularemia associated with group consumption of infected food products.

Measures against Infection in Industries which Process Agricultural Products. Such cases can occur unexpectedly in the midst of complete epizootic welfare in the surrounding territory and even outside of the limits of the natural foci of tularemia, for example, in large cities. This is associated with the fact that the raw material may sometimes be brought in for production from far away, including from localities which at that time are having an unrecognized tularemia epizootic. In connection with this it is essential to give constant attention to the prophylaxis of occupational diseases in industry. Tularemia vaccination is compulsory for all persons coming to work at sugar, starch syrup, brewery, vegetable drying enterprises, creameries and distilleries, mills and elevators (Yu. A. Myasnikov and O. V. Ravdonikas, 1954). It is advisable to check workers in the departments which are responsible for the initial processing of raw material with the use of the tularin test for the presence of immunity before the work season begins. Giving inoculations to the workers only in the presence of a large number of rodents in the fields, which has been recommended by some authors, is unacceptable (A. P. Levchenko, 1955), because the occurrence of cases at the industry may not be connected with the epizootic situation in the region in which the enterprise is located, which has been mentioned above.

At those factories in which the raw material is first wetted chloride of lime should be added to this water according to a calculation such that in the waste water there be no less than one milligram of active chlorine per liter. At the majority of factories the raw material is subjected to thermal processing adequate for disinfection. Where there is no thermal processing in the industrial process the infected raw material, for example, grain, should be heated specially at 70° for no less than a half-hour. All production wastes should be subjected to the same processing before being taken out of the territorial limits of the plant. After the work as well as before eating and smoking the hands should be carefully disinfected, for which purpose there should be barrels containing clarified (0.1 percent) solution of chloride of lime, washstands, soap and towels.

The records of the raw material coming to the plant should have a note on them by the sanitation organs with reference to the well-being or lack of well-being of the place of shipment with regard to tularemia. In the presence of a statement to the effect that the raw material has come from a locality in which there is an epizootic or if

the bodies of rodents are found in it, it should be freed of rodents and their bodies and loaded into a ratproof room with concrete floors in which the rodent holes have been sealed up, the doors lined below with iron, with windows and vents covered with an iron screen. In the absence of such rooms it is permissible to unload the raw material on open platforms which have first been freed of rodents and are surrounded by gutter traps. The gutters are kept in good order, and the rodents falling into them are destroyed. For the purpose of carting out the raw material detachable bridges are used, taking them up after cart or truck passes. Such a strict isolation of the unfavorable raw material is necessary to avoid the spread of the epizootic among local rodents and the creation of a new tularemia focus. For these purposes the raw material infected with tularemia is processed first. By using this combination of measures we completely prevented not only disease in people but also the occurrence of a tularemia outbreak among the local rodents at one brewery, where a large batch of infected barley had been brought in which the tularemia pathogen was shown bacteriologically (Yu. A. Myasnikov, 1955).

In order to avoid secondary infection of the finished products the latter are either brought outside of the enterprise immediately or are placed in ratproof storehouses. In the enterprise itself and in the adjacent structures complete repeated deratization is carried out, particularly carefully before the beginning of the season in which the raw material is processed. For the purpose of disinfecting infected seed grain on elevators it is sufficient to keep it at a temperature above 10° for two months.

Measures against Infection at Meat Combines and Meat Canning Plants. I. F. Berezin (1931), after describing outbreaks associated with the processing of carcasses of hares at a canning plant for the first time in the Soviet literature, concluded that freezing of carcasses, work in rubber gloves, etc. are unsuitable for preventing tularemia among workers in the plants. The author saw a solution in the extensive incorporation of mechanization into production. The latter, although it does reduce the direct contact with infected hare carcasses, does not exclude infection from the infected machines or from the sprinkling of water in which the frozen carcasses are thawed out. Recently, cases have been noted associated with the slaughter of sheep and other animals at meat combines; in these animals pasture ticks infected with the tularemia pathogen were found in large numbers (R. Ya. Chernina, 1953; M. P. Tereshchenko and coauthors, 1956; S. M. Smirnov, 1956).

With the aim of prophylaxis it is essential to vaccinate all persons coming to work at the meat combines and meat canning plants.

against tularemia. A batch of meat in which the existence of the tularemia pathogen has been established bacteriologically should be subjected to thermal processing. Considering the fact that the ixodid ticks falling off the cattle can create new foci of infection a timely inspection should be made of animals coming into the meat combines and ticks found on them should be destroyed, observing precautionary measures indicated for arthropod-borne infections from ticks. Tick-infested cattle is subjected to a veterinary check for the purpose of establishing the existence of cases of tularemia. For the purpose of preventing the spread of the infection beyond the limits of the industrial enterprise the waste water of the factory should be chlorinated regularly, deratization should be accomplished regularly, and ratproofing of the premises and storehouses of the factory should be provided.

Measures against Infection in Ditches and Trenches. As was pointed out in Chapter VI, during the Second World War cases of tularemia were observed in people in trenches, blindages and ditches, which required the development and realization of appropriate prophylactic measures (V. S. Grikurov, 1946; A. I. Volkov, 1948, and others). During this period there was no tularemia vaccine as yet; therefore, measures were directed at the elimination of sources of infection and elimination of the routes of its transmission.

G. P. Rudnev (1942) was one of the first to generalize on the measures for prophylaxis of trench tularemia outbreaks. He pointed to the need for regular sanitary-epidemiological reconnaissance for timely detection of the epizootic and cases of tularemia among people, which is particularly important during the advance of troop units. These measures provided not only the prophylaxis of the trench outbreaks of tularemia themselves but also of the water-borne, food product and other types of infections frequently encountered at the front. Without going into details, which have been discussed thoroughly by T. Ye. Boldyrev (1955), G. P. Rudnev (1955), K. F. Akinfiyev (1955), N. I. Kalabukhov (1955) and others, we should like to note that rodent control was practiced extensively among the troops, dugouts were protected against them, supplies of food products and sources of the drinking water were protected, water was chlorinated, etc. with the aim of prophylaxis of tularemia. The use of straw and hay from stables infested with rodents was not permitted as litter; for this purpose it was recommended that the branches of trees, chiefly coniferous, be used. The consumption of water from open water bodies which had not been disinfected and from wells which had not been inspected as well as the utilization of prepared food given by the population was prohibited.

### Measures for the Sanitization of Natural Foci of Tularemia

(This section was written by N. G. Olsuf'yev)

At the beginning of the study of tularemia all animals which were found naturally infected with the tularemia pathogen were categorized as "sources" and "vectors" of the infectious disease without a proper experimental check (B. V. Voskresenskiy, 1943). Because the number of species of such animals was very great and with further study tularemia rapidly increased, the impression of the impossibility of sanitization of natural foci was created. However, subsequent investigations made it clear that the maintenance of these foci is, by and large, assured by only a few species of rodents which are highly sensitive to this infectious disease (group I), and of the vectors, chiefly the ixodid ticks (see Chapter V). Because of this, sanitization of natural foci showed promise.

Of the six types of natural tulermia foci which have been described at the present time the most active (with respect to possible epidemiological manifestations) are the following: soddy-alluvial-boggy, meadow-field, steppe, and foothill-brook. Specifically in the foci of these types the greatest number of cases have been observed among people, and here the combination of prophylactic measures, chiefly vaccination is accomplished most methodically.

Breaking the interrelationships between members of the biocoenosis in which the disease pathogen is included, a break in which the epizootic chain is cut, should be made the basis of sanitization of the natural foci of the infectious disease. Naturally, the destruction of the continuity in contact between pathogen, vector and warm-blooded reservoir should lead to a subsidence of the focus and sterilization of the locality (Ye. N. Pavlovskiy).

Study of the characteristics of tularemia epizootology shows that for the purpose of providing for the existence of the tularemia microbe in a natural focus a tremendous part is played by masses of rodents which appear periodically -- the main sources of the infectious disease -- and ixodid ticks, its vectors. There is every reason to believe that there is no need to exterminate wholly (that is, as a zoological species) the main sources of the infectious disease and its vectors in order to break the epizootic chain and sanitize the focus. The problem should be reduced to bringing their censuses to a level which excludes the possibility of their appearance here en masse. For this purpose the entire combination of measures presented above should be used.



In foci of the soddy-alluvial-boggy and foothill-brook types the main attention should be given to a considerable and persistent reduction in the water rat census. According to existing data the active yearly occupation centered around this animal can, to some degree, localize these foci (N. G. Olsuf'yev and coauthors, 1958, 1959). Improvement operations in river valleys, boggy localities, etc., which destroy the habitats of the water rat as well as of mosquitoes, horse-flies and other vectors may also be of great importance. In foci of the meadow-field and steppe types measures should be directed at the prevention of mass multiplication of the common vole and house mouse. This can be achieved by an active development of agrotechnics, through cultivation of fields and further mechanization of harvesting operations, with constant regular extermination of rodents in the survival areas. In all the foci listed the control of ticks -- vectors and reservoirs of the tularemia microbe -- should be conducted as an obligatory matter. Thereby, it is important to bring about a marked reduction in the census of ticks in the microfoci of the infectious disease.

In a number of oblasts of the temperate belt of the European portion of the USSR as well as in places in the south a reduction of the activity of foci of the meadow-field and steppe types has been noted in recent years, and in particular there has been an absence of any particularly large increases in the censuses of small mouse-like rodents (of the "mouse invasion" type) and of diffuse tularemia epizootics associated with these rises. This undoubtedly should be related to the considerable reinforcement of agriculture as a whole, the intensification of agrotechnical measures, the increase in the plowing of virgin and wastelands, etc., which have caused a deterioration in the existential conditions of small rodents. In places, for example, in Stavropol'skiy Kray, where the mass destruction of ixodid ticks on cattle, being conducted by veterinary workers with the aim of controlling hemosporidiosis has also exerted a suppressive effect on the natural tularemia foci (A. A. Zaytsev).

The examples described indicate the real possibility of sanitization of natural foci of tularemia. However, this matter needs further study. The resolution of the problem of sanitizing natural foci of tularemia depends on the degree of utilization of the methods proposed by kolkhozes, sovkhoses and other organizations and enterprises, and is a problem of the immediate future.

**Departments of Particularly Dangerous Infectious Diseases of Oblast (Kray or Republic) Sanitary-Epidemiological Stations and Their Part in the Organization and Control of Measures against Tularemia**

The characteristics of tularemia, which have been mentioned at the beginning of the Chapter, which make difficult its control and prophylaxis, the newness and the fact that the infectious disease has been little studied have led to the fact that during the first few years after tularemia was found in the Soviet Union public health organs in a number of cases appeared in the role of "recorders" of the facts, and the epidemic outbreaks which occurred concluded naturally at the end of the epizootic. The high degree of contagiousness of the pathogen, the massiveness of the lesions, and the duration of the cases required including tularemia in the group of particularly dangerous infectious diseases. Some medical men erroneously believed the cause of this was the fact that "tularemia has in the past been confused with plague" (I. Ya. Serebriyskiy, 1948). The high degree of contagiousness of the pathogen required creating special laboratories with a strict routine and with well-trained personnel. For the purpose of demonstrating the dynamics of the rodent census -- the sources of the infectious disease -- the organization of a zoological service was required which previously had been almost unknown by sanitation organs in the center of the European portion of the USSR and West Siberia. After a large epizootic in a number of oblasts in 1938, accompanied by considerable morbidity among people, the need for creating such a new antiepidemic institution -- the tularemia (tularemia-control) station -- was demonstrated in a particularly striking manner. In 1938, in the most unfavorable regions the first tularemia stations were created (Zaraysk and Podol'sk, later in the village of Semenovskoye in Mikhnevskiy Rayon of Moskovskaya Oblast; Plavsk in Tul'skaya Oblast; Borisoglebsk of Voronezhskaya Oblast; Mikhaylof of Ryazanskaya Oblast).

The tularemia stations first did their work like plague-control institutions but soon the unsuitability of such copying became clear. The differences in the biology of the rodents -- the main sources of plague and tularemia -- required different methods of recording and extermination and also were responsible for differences in other antiepidemic measures.

During the years of the Second World War tularemia was found in many new oblasts which for the most part had been under temporary occupation. While in 1941 it was recorded in 16 oblasts, in 1943 it was found in 25; in 1945, in 31 oblasts. Numerically cases increased by 18.3 times in 1945 by comparison with 1941 (I. G. Akimov, 1946). In connection with this, in the oblasts unfavorable with respect to tularemia oblast tularemia stations were opened in 1943 which were to be in charge of the entire tularemia-control work in the oblast.

In the post-War years, in addition to the RSFSR, tularemia-

control stations were opened in a number of union republics, where there were enzootic tularemia foci. The introduction of vaccination into antiepidemic practice, the expansion of parasitological work and other changes which took place during these years made it necessary to take a new approach to tularemia-control institutions. In 1948 the Ministry of Health USSR approved a new "Statute on the Tularemia-Control Station", which defined the stations as special practical scientific institutions designed for work in regions unfavorable with respect to tularemia. During the work of the stations considerable material was accumulated requiring analysis and generalization, because the manifestations of the infectious disease on the territory of each oblast showed differences, and elucidation of them by the personnel of the scientific institutions only would hold back the detection of the characteristics of this infectious disease for a long time. In addition, new effective approved prophylactic agents were necessary, and first of all it was essential to establish the degree of effectiveness of tularemia vaccination. All this made it necessary to impute a practical scientific character to the tularemia-control stations. Of great importance in standardizing the method of operation of the tularemia-control stations were the "instructions on methods of epidemiological, zoological, parasitological and bacteriological work of the tularemia-control station", made out by N. G. Ol'suf'yev and V. V. Kucheruk, approved in 1952 by the Ministry of Health USSR. (Tularemia. Organizational-Methodological Materials, pages 23-113). These instructions successfully combined the achievements of science in the study of tularemia with the experience of practical medical institutions and assisted in enhancing their work.

In the post-War years the tularemia-control stations were strengthened organizationally and materially and carried out the control of tularemia and its natural foci successfully. In three years only (from 1949 through 1951) the morbidity rate from tularemia had been reduced by eight times in the RSFSR (V. I. Vashkov and Ye. A. Pronin, 1955), and in various oblasts, during years which were equally with respect to the epizootic status, the morbidity rate was reduced by 10 times (V. S. Sil'chenko, 1953; Yu. A. Myasnikov, 1953). The tularemia-control conference in 1953 noted in its resolution that "in those oblasts where there are no tularemia-control stations, tularemia-control measures are being organized and realized much less effectively, while there is no observation of the rodent census dynamics at all".

The work experience of tularemia-control stations showed that these institutions, in their majority, successfully combined scientific with practical work, directing their efforts toward a reduction

in the incidence of tularemia. A particularly great contribution to science was made by tularemia-control stations in matters of studying regional epidemiology, natural tularemia foci, immunological and epidemiological effectiveness of tularemia vaccination, further development of methods of laboratory diagnosis of tularemia, clarification of the species composition of rodents, ticks and other vectors in various regions, etc.

In 1955 by order of the Ministry of Health USSR the tularemia-control stations were combined with brucellosis stations and reorganized into departments of particularly dangerous infectious diseases of the oblast, kray and republic sanitary-epidemiological stations. These departments operate under a common methodological supervision from the plague-control institutions. The scientific center for tularemia is the laboratory of tularemia of the department of infectious diseases with natural foci of the IEM imeni Gamaleya of the Academy of Medical Sciences USSR.

At the present time, departments of particularly dangerous infectious diseases are doing their work in tularemia in four main divisions: epidemiological, zoological, parasitological and bacteriological. The content of work in each division has been presented in detail in the "Instructions on Methods" mentioned above. We should like to note only that the building in which the department may be located should be appropriate to a routine of work with microbes of group I. Particularly great attention should be given to preventing the spread of infectious disease beyond the laboratory confines. Because the departments of particularly dangerous infectious diseases are the only practical public health institutions which have mastered the method of investigating zoonotic infectious diseases, in recent years, aside from investigations of tularemia, brucellosis and anthrax, they have had to occupy themselves with progressively greater frequency in studying other diseases with natural focalizations: listerellosis, leptospirosis, etc. Departments of particularly dangerous infectious diseases should hold consultations with the local veterinary laboratory, bacteriological and parasitological departments of the oblast sanitary-epidemiological station, the Institute of Epidemiology and Microbiology, the national forest committee, the local society for regional study, the society of hunters, and other institutions and organization. In the rayons it is essential to create a system of correspondence made up of a group of teachers, agronomists, hunters, medical workers, who should inform the department of any increase in the rodent census within their village councils and the finding of deaths among them.

On the basis of the comprehensive plan of tularemia control measures the department develops in detail the yearly and quarterly

plans of operation. For certain seasons the calendar plans of operation of visiting work can be made up (for example, for the tick season, seasons of spring and autumn rodent census records, the hunting season, season of arthropod-borne outbreaks, etc.).

At the present time, the departments of particularly dangerous infectious diseases of oblast, kray and republic sanitary-epidemiological stations are confronted with important and significant problems with respect to tularemia: 1) the planning and regular control of the course of vaccination and revaccination of the population against tularemia in enzootic regions; 2) selective checking of the immune segment among the population with the aim of maintaining it on a high level, assuring the elimination of cases of tularemia; 3) timely formulation of prognoses and detection of epizootics among rodents; 4) observation of the accomplishment of other measures providing for the prophylaxis of tularemia; 5) detection of latent foci of tularemia by the method of bacteriological examination of pasture ticks and other methods; 6) further development and testing of methods of eliminating natural foci of tularemia; 7) the application of experience in tularemia-control work to the study and elimination of other diseases with natural focalization; 8) scientific research work on problems stemming from the practical problems of the department.

### Conclusion

On the basis of a complete study of tularemia Soviet investigators have worked out highly effective methods for the prophylaxis of tularemia, which are now being applied extensively in foci of this infectious disease in the form of a definite system of measures.

As an important achievement it may be pointed out that in the USSR, as the result of mass vaccination conducted in combination with other measures, the yearly incidence of tularemia in the past nine years (1951-1959) has been reduced by an average of 21 times compared with the figures for the previous six years (1945-1950). The main part in carrying out successful prophylaxis of tularemia has been played by specialized institutions (tularemia-control stations, which have now been reorganized into departments of particularly dangerous infectious diseases of the oblast, kray and republic sanitary-epidemiological stations), which from now on are to be in charge of this work. A problem for the future is the complete elimination of tularemia in people in the Soviet Union. This problem can be solved successfully by means of the extensive application of approved methods of tularemia prophylaxis, including methods of sanitization of the natural foci of this infectious disease.

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